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Original Article

COMPARISON OF VOLATILE COMPOSITION OF SUPERCRITICAL CARBON DIOXIDE EXTRACT FROM RHIZOMES OF KOREAN MEDICINAL PLANT 'CHUN-KUNG' (*CNIDIUM OFFICINALE* MAKINO) BY DIRECT-AND SPME-GC/MS

BO-RAM YEON[1], KANDHASAMY SOWNDHARARAJAN[1], JI-WOOK JUNG[2], JIN-WOO JHOO[3], SONGMUN KIM[1]*

[1]Department of Biological Environment, Kangwon National University, Chuncheon 200701, Republic of Korea, [2]Department of Herbal Medicinal Pharmacology, Daegu Haany University, Kyungsan, Republic of Korea, [3]Department of Animal Products and Food Science, Kangwon National University, Chuncheon 200701, Republic of Korea.
Email: perfume@kangwon.ac.kr

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ABSTRACT

Objective: The main purpose of this study was to evaluate the volatile composition of supercritical fluid extract (SFE) obtained from *Cnidium officinale* Makino rhizomes.

Methods: GC/MS analyses were carried out with the direct- and solid phase microextraction (SPME) of SFE obtained from rhizomes. In addition, GC/MS analysis was performed for the rhizomes of *C. officinale* using SPME.

Results: SPME-GC/MS analysis of the rhizomes revealed the separation of 23 components. Among these, β-phellandrene (20.38%), dictyotene (12.98%), β-pinene (10.59%), β-selinene (9.45%), eugenol (7.71%) and β-farnesene (7.09%) were found to be major components. In the SFE analyzed by direct-GC/MS, linoleic acid (19.26%), 2-methoxy-4-vinylphenol (18.98%), hexadecanoic acid (12.15%), and β-selinene (9.44%) were identified as major components. Whereas, 3,4-dihydrocoumarin (16.94%), shyobunone (14.07%), dictyotene (10.65%), *p*-cresol (10.17%), zierone (6.36%) and umbellulone (5.71%) were major components in the SFE analyzed by SPME-GC/MS.

Conclusion: The present study clearly suggested that the SPME-GC/MS analysis of SFE provided the separation of more number with diverse groups of compounds than the direct-GC/MS.

Keywords: *Cnidium officinale*, Gas chromatography, Rhizome, Solid phase microextraction, Supercritical carbon dioxide extract

INTRODUCTION

Essential oils are secondary metabolites of aromatic plants and they can be biosynthesized by all of the plant parts. They are complex mixtures of volatile compounds characterized by a strong odor and have a wide range of chemical composition. Previous studies have reported that essential oils showed anticancer, anti-inflammatory, antiphlogistic, antiviral, antimicrobial and antioxidant properties [1]. They contain a variety of bioactive molecules such as terpenes, terpenoids, phenol-derived aromatic components and aliphatic components [2]. Essential oils and their components are gaining increasing interest because of their use in food, cosmetics and pharmaceutical industries, it is necessary to investigate the components of the essential oil of the plants.

Hydrodistillation is a conventional technique extensively used to extract essential oils from aromatic plants, but this method has several disadvantages including hydrolysis or thermal degradation of the most sensitive compounds and affects the quality of the essential oil [3,4]. Supercriticalfluid extraction (SFE) is one of the alternative techniques with carbon dioxide (CO_2) as a supercritical fluid, it allows shorter extraction times and thus preserving the original composition of the extracted volatiles [4,5]. Various analytical procedures have been used to identify the volatile components from the plant source. Among these extraction techniques, solid phase microextraction (SPME) combined with GC/MS method is a novel approach, solvent-free, rapid and very simple to operate for the extraction of organic compounds [6]. The technique assimilates extraction, concentration and sampling into a single step. Recent studies have revealed that the fiber coated with non-polar, semipolar and polar adsorbing phase was more appropriate for analysis of essential oil composition [7]. Mesomo et al. [8] studied the composition of SFE and essential oil of *Zingiber officinale* R. and reported that the chemical profiles were found to be similar for the two methods; however, the quantities of the components extracted were different. SPME provides many advantages over conventional sample preparation techniques. The volatile substances are concentrated on an absorbing layer, transferred directly to the injection port and separated in the chromatographic process.

Cnidium officinale Makino is a perennial plant, belonging to the Apiaceae (Umbelliferae) family and widely distributed in Korea, China and Japan. This plant is commonly called as 'Chun-Kung' in Korea. The roots of *C. officinale* are useful as a component of liquor, and its leaves are used in salads and as a condiment [9]. Traditionally, the plant is used for the treatment of various disorders, especially the dried rhizomes of this plant is used to treat pain, inflammation, menstrual disturbance, and vitamin deficiency diseases. Several studies have reported that they possess pharmacological properties such as tumor metastasis, angiogenesis and act as an inhibitor of high glucose-induced proliferation of glomerular mesangial cells [10–12]. Furthermore, previous reports suggested that the *C. officinale* has blood circulation and inflammatory disease regulatory properties [13]. Previously, Choi et al. [9] reported the essential oil composition of *C. officinale* rhizomes by using steam distillation and identified 68 different components. Some authors have studied that the essential oil composition of leaves and rhizomes of *C. officinale* extracted by steam distillation and showing wide variation in the chemical composition [9,14,15]. Bae et al. [16] studied the chemical composition of rhizome extracts and evaluated their biological effects under *in vitro*. Based on the above knowledge, the aim of the present work was to compare the volatile components of rhizomes of *C. officinale* obtained from SFE by two different methods such as direct and SPME followed by GC/MS analysis. Further, SPME-GC/MS analysis of rhizomes was also determined.

MATERIALS AND METHODS

Plant material

Rhizomes of *Cnidium officinale* Makino 'Chun-Kung' were purchased from BN Herb Inc., Pyeongchang, South Korea. A voucher specimen was deposited in the Herbarium of Kangwon National University

under the number KWNU80092. The plant material was dried at room temperature, ground to a powder using a blender and stored at −20 ºC prior to use.

Supercritical CO_2 extraction

The supercritical CO_2 extract (SFE) of *C. officinale* rhizomes was performed on an ISA-SCCO-S-050-500 SFE device (ILSHIN Autoclave Co. Ltd., Daejeon, Korea). Carbon dioxide (99.5%, w/w pure) was delivered from a standard cylinder and compressed to an extraction pressure by an air-driven liquid pump. For each condition, about 120 g of milled *C. officinale* rhizomes were loaded into a stainless steel extraction vessel. During the extraction process, the pressure, temperature and CO_2 flow rate were controlled by adjusting the regulating valves. To optimize the SFE conditions for *C. officinale* rhizomes, the extraction was conducted at different pressures (200, 300 and 400 bar) and temperatures (40, 50, 60 and 70°C). The CO_2 flow rate was maintained at 30 mL/min. After one hour of extraction time, the extraction vessel was depressurized and the oil was collected from the separation vessel. The amount of extracted oil was determined gravimetrically after collection, and the extraction yield was expressed as percent of the dry weight of *C. officinale* rhizomes.

SPME conditions

The air dried rhizomes of *C. officinale* were cut in to small pieces as 1–2 cm long before subjection to SPME. Two grams of rhizomes were introduced into a 20 mL SPME vial. For the extract obtained from SFE, 1 mL of SFE was introduced into SPME vial. The SPME device coated (fused-silica fiber) with a 100 µm layer of polydimethylsiloxane (Supelco, Bellefonte, PA, USA) was used for extraction of the plant volatiles and the vial was sealed with a silicone septum. They were exposed in the SPME vial at 60°C for 30 min and immediately introduced in the gas chromatography injector.

Gas chromatography/mass spectrometry (GC/MS) analysis

GC/MS analysis was carried out with the direct injection and SPME of SFE extracted from rhizomes. In addition, GC/MS analysis was performed for the rhizomes of *C. officinale* using SPME. For this purpose, GC/MS analysis was performed using a Agilent 7890A/ Agilent 5975C gas chromatography/mass spectrometer, equipped with a HP-5MS (30 m × 0.25 mm, 0.25 µm film thickness, Agilent, USA) fused silica capillary column. The oven temperature was initially at 50°C (held for 5 min) and then increased to 25°C at 5 ºC/min. The injector temperature was maintained at 250 °C. The ion source temperature was 280°C and electron energy was 70 eV. The analyzer was scanned over the range of m/z 50 to 500. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. The components of rhizomes and SFE were identified by their linear retention indices relative to the series of *n*-alkanes as well as by comparing of their mass spectra and retention times with Wiley 275, NIST 3.0 and Adams spectral libraries [17].

RESULTS AND DISCUSSION

Extraction yield of SFE

The essential oil of plants is generally extracted by steam distillation or solvent extraction. Based on the extraction method used, the essential oil composition may change leading to variations from the natural fragrance of the plant. Several advanced techniques have been introduced for the extraction of essential oils from plants in order to shorten the extraction time, decrease the solvent consumption, increase the extraction yield and enhance the quality of essential oils. Among the various methods, the supercritical fluid extraction (SFE) has attracted considerable attention in the past decades as an effective and environmentally friendly method to replace conventional methods [18,19]. In the SFE method, carbon dioxide is one of the most commonly used supercritical fluids because it is non-flammable, fairly non-toxic, cost-effective, odorless, readily available solvent and easily removed from the extract [20]. Hence, SFE was performed for the extraction of volatile components from rhizomes of *C. officinale*. Subsequently various parameters possibly influence the extraction yield, the optimization of the experimental conditions denotes an essential step in the development of a SFE method. The yields of SFE from the rhizomes of *C. officinale* are depicted in Fig. 1. For optimizing the SFE extraction conditions of the essential oil from rhizomes of *C. officinale*, extraction was performed at different temperature (40, 50, 60 and 70°C) and pressure (200, 300 and 400 bar). The influence of four different temperatures on the SFE from *C. officinale* rhizomes was studied using a constant pressure of 400 bar. The results indicated that the significant increase of SFE yield with increase of temperature from 40°C to 70°C (0.5 – 2.1%) at constant pressure of 400 bar. The influence of pressure was studied using a constant temperature at 70°C based on the higher yield. The yield was increased while decreasing the pressure from 400 to 300 bar (2.1 to 4.1%), but the yield was decreased with further increment of pressure at 300 to 200 bar (4.1 – 2.8%).

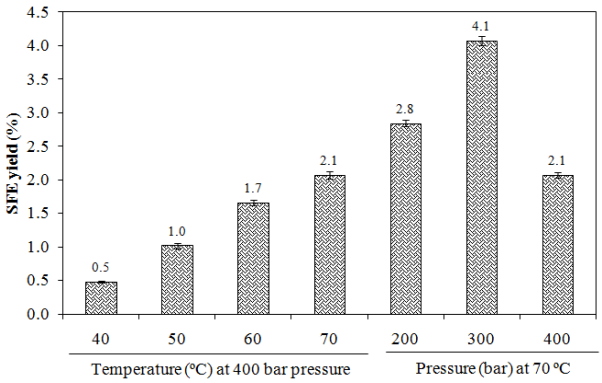

Fig. 1: Yields of SFE from rhizomes of *Cnidium officinale* at different extraction conditions. Values are mean of three replicate determinations (n= 3) ± standard deviation.

In the present study, it was observed that the temperature and pressure are the most important parameters that influence the extraction yield and the composition of SFE. Based on the findings, we found that the optimized extraction conditions for the yield of SFE (from *C. officinale* rhizomes) are temperature at 70°C and pressure at 300 bar. A number of authors have reported that the SFE has been used widely for the extraction of volatile oils from aromatic plants and resulted in higher yields. They suggested that the temperature and pressure are the important parameters that highly influenced the yields of SFE [4,5,20,21].

Chemical composition of rhizomes and SFE

The chemical composition of rhizomes and SFE of *C. officinale* was determined by GC/MS (Table 1). In GC/MS analysis, the composition of SFE was compared with direct- and SPME-GC/MS. The rhizomes of *C. officinale* revealed the separation of 23 components in its composition by SPME-GC/MS. β-Phellandrene (20.38%), dictyotene (12.98%), β-pinene (10.59%), β-selinene (9.45%), eugenol (7.71%), β-farnesene (7.09 %) and 3,4-dihydrocoumarin (7.07%) are the major components in the rhizomes. The presence of the major compound β-phellandrene was reported in various aromatic and medicinal plants including *Angelica purpuraefolia*, *A. archangelica*, *Chaerophyllum aromaticum* [22–24]. In the SFE, 19 components were detected for direct/GC-MS and 30 components were identified for SPME-GC/MS. Among the various groups of components, more numbers of compounds were identified under the group of hydrocarbons (Table 2).

The rhizomes registered higher concentration of hydrocarbon group compounds (78.28%) followed by direct-GC/MS analysis of SFE (36.95%) and SPME-GC/MS analysis of SFE (30.47%). 2-Methoxy-4-vinylphenol (18.98%), linoleic acid (19.26%), n-hexadecanoic acid (12.15%), β-selinene (9.44%), and β-farnesene (7.33%) are major components in the SFE by direct-GC/MS. In SPME-GC/MS, 3,4-dihydrocoumarin (16.94%), shyobunone (14.07%), dictyotene (10.65%), *p*-cresol (10.17%), zierone (6.36%), and umbellulone (5.71%) are found to be major components in the SFE.

Table 1: Chemical composition of rhizomes and its SFE from *Cnidium officinale* by direct - and SPME-GC/MS analyses

Compound name	RI[a]	Area (%)			Group
		Rhizomes	SFE - Direct	SFE - SPME	
α-thujene	930	0.72	-	-	Hydrocarbon
α-pinene	939	2.4	-	-	Hydrocarbon
Sabinene	975	-	-	0.21	Hydrocarbon
β-pinene	979	10.59	0.65	-	Hydrocarbon
Myrcene	990	-	-	0.14	Hydrocarbon
β-cymene	1024	0.21	-	-	Hydrocarbon
β-phellandrene	1029	20.38	0.98	-	Hydrocarbon
β-ocimene	1050	1.15	-	-	Hydrocarbon
Dictyotene	1155	12.98	3.83	10.65	Hydrocarbon
γ-terpinene	1059	0.82	-	-	Hydrocarbon
Umbellulone	1171	-	-	5.71	Ketone
p-cresol	1076	-	-	10.17	Alcohol
Geraniol	1252	-	-	0.16	Alcohol
2-methoxy-4-vinylphenol	1309		18.98		Alcohol
Eugenol	1359	7.71	0.32	-	Phenol
Copaene	1376	-	-	0.14	Hydrocarbon
3,4-dihydrocoumarin	1380	7.07	-	16.94	Coumarin
β-cubebene	1388	0.76	1.35	0.47	Hydrocarbon
β-elemene	1390	2.23	2.82	3.99	Hydrocarbon
Vanillin	1394	-	0.56	-	Aldehyde
Italicene	1405	2.62	2.66	0.95	Hydrocarbon
α-gurgujene	1409	-	-	0.58	Hydrocarbon
α-cedrene	1411	0.6	0.97		Hydrocarbon
β-caryophyllene	1419	2.61	2.00	-	Hydrocarbon
β –gurgujene	1433	-	-	0.34	Hydrocarbon
γ-elemene	1436	-		0.46	Hydrocarbon
β-farnesene	1442	7.09	7.33	2.99	Hydrocarbon
α-caryophyllene	1454	0.45	0.54	1.19	Hydrocarbon
α-acoradiene	1466	0.65	1.15	0.40	Hydrocarbon
γ-himachalene	1482	-	-	0.14	Hydrocarbon
β-selinene	1490	9.45	9.44	-	Hydrocarbon
cadine-1,4-diene	1495	0.47	-	0.42	Hydrocarbon
α-selinene	1498	2.1	3.23	1.38	Hydrocarbon
Shyobunone	1506		-	14.07	Ketone
cycloisolongifolol	1513	-		0.57	Alcohol
β-cadinene	1523	-	-	1.68	Hydrocarbon
Calamenene	1529	-	-	3.38	Hydrocarbon
Calacorene	1545		-	0.96	Hydrocarbon
Zierone	1575	-	-	6.36	Ketone
alloaromadendrene oxide	1641	-	-	4.14	oxide
butylphthalide	1648	1.43	-	2.41	Phthalide
Asarone	1676	-	-	1.95	Ketone
cedr-8-en-13-ol	1689	-	-	0.57	Alcohol
3-butylidenephthalide	1718	0.35	-	--	Phthalide
hexadecyl alcohol	1875	-	2.80	-	Alcohol
hexadecanoic acid	1960	-	12.15	-	Acid
linoleic acid	2133	-	19.26	-	Acid

RI[a], the retention index published by Adams (2007). Rhizome was analyzed by SPME-GC/MS.

Table 2: Percentage of different groups of compounds detected from rhizomes and its SFE of *Cnidium officinale* by direct - and SPME-GC/MS analyses.

Group of compounds	Rhizomes	SFE - direct	SFE - SPME
Hydrocarbon	78.28	36.95	30.47
Alcohol	-	21.78	11.47
Ketone	-	-	28.09
Coumarin	07.07	-	16.94
Acid	-	31.41	-
Oxide	-	-	04.14
Phenol	07.71	00.32	-
Phthalide	01.78	-	02.41
Aldehyde	-	00.56	-
Total	94.84	91.02	93.52

Rhizome was analyzed by SPME-GC/MS.

Choi et al. [9] studied the essential oil composition of *Cnidium officinale* rhizomes by using steam distillation. A total of 68 compounds were identified by capillary GC/MS analysis. In this, 25 hydrocarbons (15.9%), 8 aldehydes (0.7%), 13 alcohols (11.5%) and three ketones (0.5%) were detected. *cis*-Butylidene phthalide (33.2%), 3-butyl phthalide (21.1%), *cis*-3-isobutylidene phthalide

(10.1%) and terpinen-4-ol (8.5%) were the main volatile components of *Cnidium officinale* rhizomes. Whereas, in the present study revealed that the β-phellandrene (20.38%) and 3,4-dihydrocoumarin (16.94%) were the major components in the rhizomes and its SFE using SPME, respectively. Moreover, phthalide compounds constitute only 2.41%, which was reported by Choi et al. [9] as major components. Several authors have reported that the variations in the chemical composition of volatile components may differ according to the season, geographical origin, extraction methods and analytical techniques [2,25,26]. From the results, the main components detected from the rhizomes and its SFE are quantitatively and also qualitatively different. In the present investigation, eight components such as α-caryophyllene (0.45 – 1.19%), dictyotene (3.83 – 12.98%), β-farnesene (2.99 – 7.33%), α-acoradiene (0.40 – 1.15%), italicene (0.95 – 2.66%), β-cubebene (0.47 – 1.35%), β-elemene (2.82 – 3.99%) and α-selinene (1.38 – 3.23%) were identified in both of the rhizomes and its SFE of direct and SPME with major fluctuations. The results of the present study clearly indicated that the SPME analysis of SFE provided the separation of more number of compounds with diverse groups than the direct injection method. In our experiment, fiber coated polydimethylsiloxane was used to analyze the SFE components of rhizomes of *C. officinale*. Recent studies have reported that the interest in using SPME for volatile oil analysis in food and pharmaceutical industries has been increased during this decade. SPME is a simple, rapid and most useful sample preparation method for the determination of essential oil components from aromatic plants [27]. SPME analysis allowed a qualitative estimation of volatile compounds using a small quantity of sample [28].

CONCLUSION

Based on the results, it could be concluded that the SPME has been provided the better separation of SFE components from the rhizomes of *C. officinale* and more efficient than the direct injection method. Further, SPME allows rapid extraction and could be appropriate for the analysis of volatile components from plants.

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CONFLICT OF INTERESTS

Declared None

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