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Extraction of Each Part of *Chamaecyparis obtusa* Using Supercritical CO_2

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Abstract

In this study, extraction of *Chamaecyparis obtusa* oil from leaf, wood, bark, cone using supercritical CO_2 was carried out. The highest yield was obtained from leaves. The supercritical CO_2 extraction of C. obtusa was influenced by temperature. When the temperature increased from 40℃ to 50℃ and 60℃, the yield increased. Among various parts of C. obtusa, the cone showed higher temperature dependency on extraction yield and rate than other parts. The color of oils extracted was different. The oil color from leaves was deep green. The oil from wood was brown red. Oil colors from bark and cone were yellow and brown respectively.

Keywords: ***Supercritical fluid extraction; CO_2; Chamaecyparis obtusa***

Introduction

Phytoncides from *Chamaecyparis obtusa* has been reported to have many biological activities including antioxidant, antimicrobial activity, and NK cell activating activity [1,2]. However, there are not many evidences about the effects of phytoncide on the cardiovascular diseases [3]. There are many kind of secondary metabolism components from *C. obtusa* such as 66% kind of monoterpene and 25% sesqui-terpene. [4]. These compounds are attributed many physiological effects, such as antioxidant and anti-mutagenic activities [5]. In the measurement of generation of cytokine of MCP-1, IL-6 and IL-8 which is of inflammatory Cytokine of EoL-1 and THP-1 cells the leaf extract injected group showed significant decrease for EoL-1 and THP-1 in compare is on with that of mite Atopic dermatitis group [6]. Many methods are available to extract oils or lipids from *C. obtusa*. The conventional method of cold compression is used to produce *C. obtusa* extract with high quality however, the yield is too low [7]. The method using organic solvent is easy and fast. However, the purity is low and there is the risk of organic solvent residue. Supercritical fluid extraction (SFE) is a promising alternative method to replace conventional solvent extraction because the supercritical CO_2 is nontoxic, nonexplosive, and easily removable from products. Recent years, SFE technology has been widely applied to wide area of industry, especially in pharmaceutical, nutraceutical and cosmetic industry. This method has several advantages over conventional extraction methods such as lower extraction temperature, lower degradation of heat-susceptible compounds. SFE has higher selectivity and easily adjust the selectivity by changing the pressure and temperature. Other advantages are it can get the high purity products, no-solvent residues because the extraction solvent commonly used is CO_2 that is inert, non-toxic and low cost [8]. The supercritical fluid extraction will be widely used by replacing conventional methods for high quality products in many industrial areas [9]. In this study, we investigated the supercritical fluid extraction of different parts of *C. obtusa* and optimized various factors influencing extraction. Their yields of oil were also compared to yields extracted with organic solvent.

Material and Methods

Materials

Plant materials: The parts of *Chamaecyparis obtusa* (leaf, wood, bark, and cone) were obtained from a grove in Jangseong-gun, Jeollanam-do Korea. The raw material was powdered, dried to get the humidity less than 5%.

Instruments: Supercritical fluid extraction system MSFE0500R1 – SC Labs in Korea. Extraction tank volume was 2,000 ml; separation tank volume was 1,000 mL, CO_2 recycler.

Methods

1. The extraction methods using organic solvent: 20 g the part of *C. obtusa* powder extracted in soxlhet system with hexane as solvent at 60℃ for 10 hours. The extract was evaporated under reduced pressure; remove the organic solvent by CO_2 continuously for 1 hour. Centrifuge at the rate of 5000 rpm, collected the liquid oil, weigh and determine the general indices of *C. obtusa* oil.

2. Supercritical fluid extraction method: Put the powder into clothes pack, place into extraction tank. Set up the parameters of temperature, pressure, CO_2 flow, and turn on the cooling system. When the set parameters were achieved, open the valve to get liquid CO_2 from containing tank flow through the cooling system and then pump up to get high pressure. CO_2 was conducted through the heating part to increase temperature, at that time liquid CO_2 will transform to supercritical state and go to extraction tank. At the extraction tank (containing raw material), the temperature and pressure are controlled to maintain the supercritical state of CO_2 according to investigated conditions. *C. obtusa* oil and other lipophilic components are dissolved in supercritical CO_2 and extracted out of raw materials then flowed to reduced pressure part before going to separation tank. The temperature and pressure of separation tank was strictly controlled, at that time supercritical CO_2 transforms to aerial state and lost the ability to dissolve *C. obtusa* oil. The *C. obtusa* oil was precipitated and taken out by the sampling part. Aerial CO_2 state was conducted to condensation tank and cooled to get the liquid CO_2 and then recycled for continuous extraction [9-11].

Results and Discussion

Extraction of each part of *C. obtusa* oil using organic solvent

Oils extracted by soxhlet extraction using organic solvent have been used in quality control or quantitative analysis of plant fats and oils. Organic solvent may be n-hexane, petroleum ether, chloroform [12]. In this study, we used the n-hexane to extract oils from various parts of *C. obtusa*. As shown in TABLE 1, different part of *C. obtusa* resulted different extraction yields. The highest yield was obtained from leaves. Some other studies reported the content of oil in leaf of *C. obtusa* in the range of 3-5%, [13]. Another different result by extraction was different colors. The color of each oil extracted was different. The oil color from leaves was deep green. The oil from wood was brown red. Oil colors from bark and cone were yellow and brown respectively.

TABLE 1. **The extraction of various parts from *C. obtusa* using n-hexane.**

	Raw material (g)	Extracted oil (g)	Yield (%, w/w)
Leaf	20	1.04	5.2
Wood	20	0.22	1.1
Bark	20	0.37	1.85
Cone	20	0.63	3.15

The supercritical CO_2 extraction of *C. obtusa*

For supercritical extraction, the carbon dioxide was compressed to 350 bar which was in supercritical condition. The flow rate was 145 ml/min. For higher extraction rate, each parts of *C. obtusa* were ground to particles sized between 800 to 1,000 µm. The temperature effect on supercritical CO_2 extraction was carried out. Extractions were made at different temperature 40℃, 50℃ and 60℃. Results were monitored according to the time as shown in TABLE 2.

TABLE 2. **The effect of temperature on supercritical CO_2 extraction yield of leaves.**

Time (min)	Yield at different temperature (%)		
	40°C	**50°C**	**60°C**
20	2.68	2.83	3.04
40	3.56	3.91	4.58
60	4.01	4.27	5.24
80	4.36	4.5	5.46
100	4.51	4.64	5.57
120	4.66	4.72	5.66

As shown in TABLE 2, the supercritical CO_2 extraction of *C. obtusa* was influenced by temperature. Different temperature resulted different yield of oil extraction. When the temperature increased from 40℃ to 50℃ and 60℃, the yield increased. At 60℃, the yield was the highest. However, the yield from the above conditions was low, at 60℃ and 120 minutes, the yield was 5.66%. In supercritical extraction, the change of temperature will change the density of supercritical CO_2, thus change its solubility [14]. In this study, we determine the yield at 40℃, 50℃ and 60℃. From the table, the yield was the highest at 60℃. The time dependent changes of yield could be used to determine the extraction rate as shown in FIG. 1.

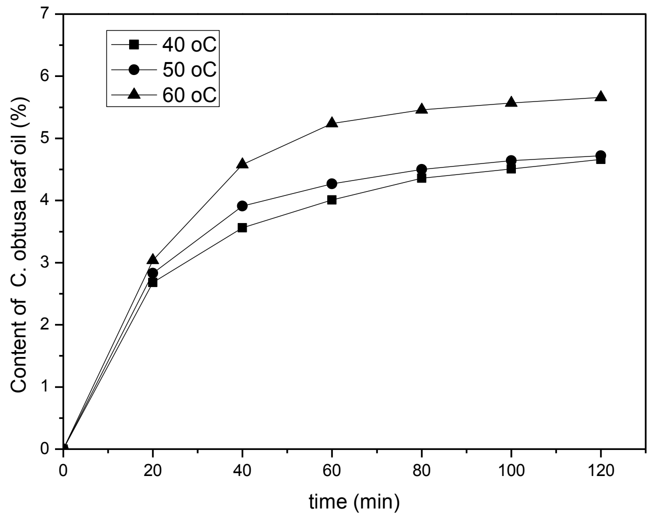

FIG. 1. **The effect of temperature on the extraction rate of leaves.**

Other supercritical CO_2 extractions were carried out with different parts of *C. obtusa*. With wood bark, same supercritical extractions were made at different temperature 40°C, 50°C and 60°C. Results were monitored according to the time as shown in TABLE 3.

TABLE 3. **The effect of temperature on supercritical CO_2 extraction yield of wood bark.**

Time (min)	**Yield at different temperature (%)**		
	40°C	**50°C**	**60°C**
20	0.33	0.54	0.59
40	0.7	0.97	0.99
60	1	1.11	1.19
80	1.16	1.18	1.27
100	1.21	1.22	1.3
120	1.25	1.26	1.33

With wood bark, different temperature resulted different yield of oil extraction. When the temperature increased from 40℃ to 50℃ and 60℃, the yield increased. At 60℃, the yield was the highest as 1.3%. The time dependent changes of yield could be also used to determine the extraction rate as shown in FIG. 2.

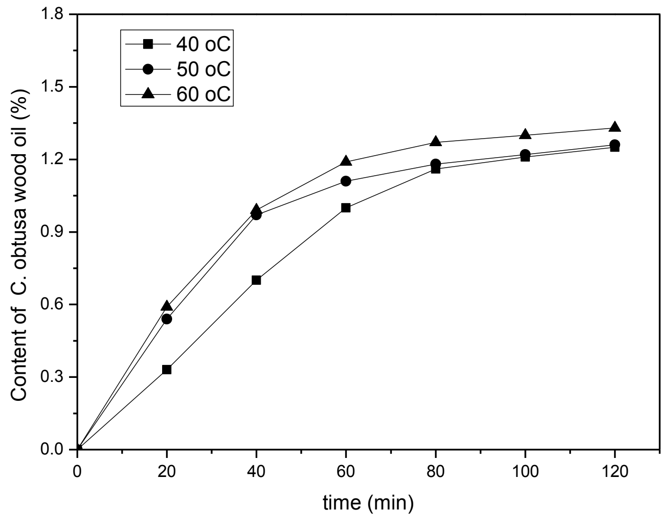

FIG. 2. **The effect of temperature on the extraction rate of bark.**

With cone, same experiments were made at different temperature 40℃, 50℃ and 60℃. Results were monitored according to the time as shown in TABLE 4. With cone, different temperature resulted different yield of oil extraction. At 60℃, the yield was the highest as 4.08%. The time dependent changes of yield could be also used to determine the extraction rate as shown in FIG. 3. With cone, the temperature dependency on extraction yield and rate was higher than other parts.

TABLE 4. **The effect of temperature on supercritical CO_2 extraction yield of cone.**

Time (min)	Yield at different temperature (%)		
	40°C	50°C	60°C
20	0.41	1.07	0.9
40	0.93	1.99	2.13
60	1.75	2.61	3.16
80	2.18	3.06	3.62
100	2.41	3.22	3.89
120	2.51	3.3	4.08

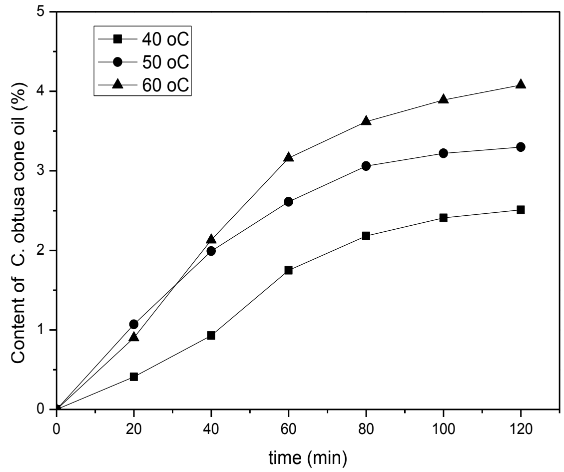

FIG. 3. **The effect of temperature on the extraction rate of cone.**

Conclusion

Supercritical CO_2 extractions were made with different parts of *C. obtusa*. Different part of *C. obtusa* resulted different extraction yields. The highest yield was obtained from leaves. The supercritical CO_2 extraction of *C. obtusa* was influenced by temperature. Different temperature resulted different yield of oil extraction. When the temperature increased from 40℃ to 50℃ and 60℃, the yield increased. At 60℃, the yield was the highest. Among various parts of *C. obtusa*, the cone showed higher temperature dependency on extraction yield and rate was higher than other parts. Another different result by extraction was different colors. The color of each oil extracted was different. The oil color from leaves was deep green. The oil from wood was brown red. Oil colors from bark and cone were yellow and brown respectively.

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