

Development of cosmetics ingredient using *Chamaecyparis obtusa* leaves originated from Jeju island extract by subcritical water extraction

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Introduction:

The purpose of this study is to develop cosmetics ingredient using subcritical water extraction (SWE) with *Chamaecyparis obtusa* leaves originated from Jeju-island and comparison of other methods of extraction. This SWE use only water and could extract active material without chemical solvent which is harmful to human's skin. In this study the antioxidant, anti-inflammatory and antibacterial effects of *Chamaecyparis obtusa* extract of Jeju island native plant using SWE was confirmed.

Chamaecyparis obtusa

Family name: Cypressidae
Distribution area: Korea (Jeju island), Japan, China
Experimental plant parts: Leaf
Folk medicine: Anti-inflammatory



Materials & Methods:

< Extraction >



< HPLC Analysis >

The surface component content analysis was measured using HPLC.

Instrument	HPLC (Waters 2695)		
Detector	PD-A detector		
Wavelength	256nm		
Column	Kromasil 100-5-C-18 5.µm 4.6x250mm (AkzoNova)		
Mobile phase	A: 0.1% formic acid in H ₂ O		
	B: Acetonitrile		
Flow rate	Time (min)	A (%)	B (%)
	0	95	5
	30	70	30
Flow rate	1 mL/min		
Injection volume	10µl		

< DPPH Inhibition Assay >

Radical scavenging activity measurement using DPPH(1,1-diphenyl-2-picrylhydrazyl) experiment method

< Anti-inflammatory >

- Cell line : Raw 264.7
- Cytotoxicity : 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay
- NO production : Griess reagent assay

< Anti-Bacterial Activity >

To measure the antibacterial activity of the sample, the size of the clear zone was confirmed by agar diffusion method. Erythromycin was used as a positive control.

- Test Microbes : Staphylococcus epidermidis (CCARM 3709) and Cutibacterium acnes (CCARM 0081)



Results & Discussion:

< Extraction Yield >

Method	Condition	Yield (% mg/g)
Solvent extraction	70% ethanol, Room temperature, 4h	14.5
Hydrothermal extraction	Water: Boiling point < 100 °C, 4h	31.5
Low-temperature extraction	Water: Room temperature, 4h	11.9
	120 °C, 60 bar, 15 min	28.1
	120 °C, 80 bar, 15 min	20.4
	135 °C, 60 bar, 15 min	25.8
	135 °C, 80 bar, 15 min	24.7
	150 °C, 60 bar, 15 min	28.2
SWE	150 °C, 80 bar, 15 min	29.1

Table1. Comparison of different methods for the extraction of *Chamaecyparis obtusa* leaves extracts and Extraction yield from various extract process of SWE

< Analysis of surface components of *Chamaecyparis obtusa* extract >

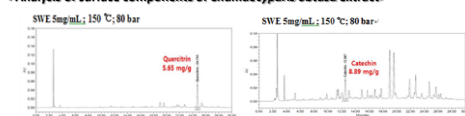


Figure1,2. Analysis of surface components of Quercetin, Catechin from *Chamaecyparis obtusa* leaves subcritical water extracts

< Anti-oxidant Effect of the SWE extract on DPPH >

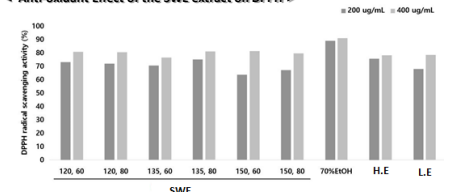


Figure 3. DPPH radical scavenging test from *Chamaecyparis obtusa* leaves extracts with various extract methods and process.

< Inhibitory Effect of Cosmetic Raw Material on Nitric oxide >

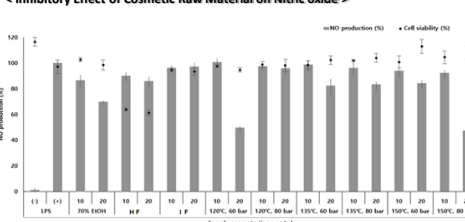


Figure 4. NO production and cell viability in LPS-stimulated RAW 264.7 cells from *Chamaecyparis obtusa* leaves extracts with various extract methods and process.

< Anti-bacterial Effect of the extract >

Method and condition	Clear zone (mm)		
	S.epidermidis CCARM 3709	C.acnes CCARM 0081	
70% EtOH Solvent extraction	10.0	11.0	
Hydrothermal extraction	8.5	-	
low-temperature extraction	-	-	
	120 °C, 60 bar	8.5	
	120 °C, 80 bar	8.5	
	135 °C, 60 bar	8.5	
	135 °C, 80 bar	8.5	
	150 °C, 60 bar	9.0	8.5
SWE	150 °C, 80 bar	9.0	8.5

Figure 5. Paper disc diffusion method from *Chamaecyparis obtusa* leaves extracts with various extract methods and process

Conclusions:

This study demonstrated that the *Chamaecyparis obtusa* leaves SWE extract inhibitory effects of inflammatory mediator, antioxidant effects, and antibacterial effects. Moreover, the extraction process using subcritical extraction equipment is relatively shorter than the extraction time using general mass production equipment. It is considered to be an economical and efficient method for extraction. Based on the results of this study, it was possible to confirm the potential of *Chamaecyparis obtusa* leaves extract as a natural cosmetic ingredient by subcritical water extraction.

Acknowledgements:

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