

# Extraction of Bioactive Components from *Centella Asiatica* Using Subcritical Water

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## ABSTRACT

*Centella asiatica* is a tropical medicinal plant with a long history of therapeutic uses, dermal disorders, venous insufficiency and microangiopathy. The substances of therapeutic interest are the saponin-containing triterpene acids and their sugar esters. Two bioactive components, asiatic acid and asiaticoside, were extracted from *Centella asiatica* using subcritical water as an extraction solvent. Extraction yields of asiatic acid and asiaticoside were measured using high-performance liquid chromatography (HPLC) at temperatures from 100 to 250 °C and pressures from 10 to 40 MPa. As temperature or pressure increased, the extraction yield of asiatic acid and asiaticoside increased. At optimal extraction condition of 40 MPa and 250 °C, the extraction yield of asiatic acid was 7.84 mg/g and the extraction yield of asiaticoside was 10.0 mg/g. Extracted asiatic acid and asiaticoside could be collected from water as particles with a simple filtering process. Dynamic light scattering (DLS) was used to characterize particle size. Particles containing asiatic acid were larger than particles containing asiaticoside, thus asiatic acid and asiaticoside can be separated each other using two different size of filters. The extraction yields of asiatic acid and asiaticoside using subcritical water at 40 MPa and 250 °C were higher than extraction yields using conventional liquid solvent extraction with ethanol at room temperature while the subcritical water extraction yields were lower than extraction yields with ethanol at its boiling point.

## INTRODUCTION

Subcritical water extraction is a technology that modifies the physical properties of water by increasing the temperature up to 374°C and by keeping the pressure high enough to maintain the water in the liquid state to improve its extraction ability. Subcritical water can be an

environmentally-friendly, non-toxic alternative for the extraction of desired polar compounds from herbs, while maintaining the naturalness of the resultant extracts. Other important advantages of subcritical water extraction over traditional are: organic liquid solvent extraction techniques require shorter extraction time, higher quality of extracts, lower solvent extraction costs, and an environmental benignity [1]. Subcritical water as an extraction solvent has been explored to extract polar, bioactive components from herbs and foods. It was shown that 80 % of oxygenate from savory and peppermint can be extracted with subcritical water at 6 MPa and 100° C [2], 54% of nutraceuticals from oregano can be extracted at 10 MPa, 200 °C [3], and 90-95 % of lignans from whole flaxseed can be extracted at 5.2 MPa and 140 °C [4].

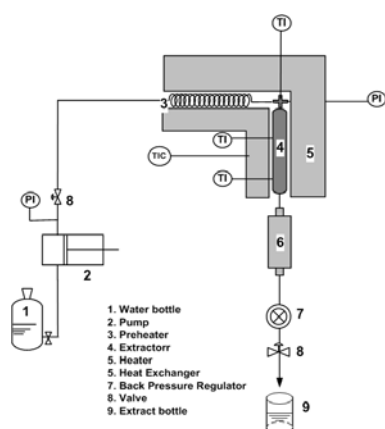
In the past, organic solvents such as methanol, ethanol, or methanol-water mixture [5] were used to obtain extracts from *Centella asiatica*. This study describes the first use of subcritical water to extract asiatic acid and asiaticoside from *Centella asiatica*. We demonstrate that the extracted asiatic acid and asiaticoside can be collected as a particle form using a simple filtering process after the subcritical water extraction. In addition, we demonstrate that the particles containing asiatic acid and the particles containing asiaticoside can be separated from each other based on their particle size. To the best of our knowledge, this is the first report that extracted components can be collected in a particle form without evaporating extraction solvent.

## EXPERIMENTAL SECTION

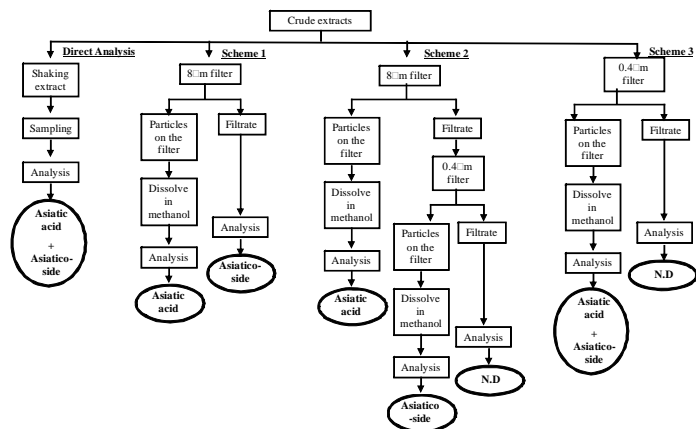
**Subcritical water extraction method.** The experiments were conducted in a laboratory-scale subcritical water extraction reactor system. A schematic diagram of the system for subcritical water extraction apparatus is shown in Figure 1. All top sections of the system were insulated in boxes of ceramic board and the temperature was monitored directly using a thermocouple. The system temperature was controlled by a temperature controller. *Centella asiatica* was introduced into a cylindrical extraction vessel with internal volume 232 mL. The solvent were pumped into the reactor system using a high-pressure pump (diaphragm metering pump, Pulsa 680, maximum flow rate = 1.2 L/h) until it reach the desire pressure. When the pressure reached at the experimentally desired pressure, the extraction vessel was heated and the static extraction was started for about 5 hr. After the extraction, water was depressurized to ambient conditions by a back-pressure regulator. The liquid extracts were collected in glass sample vials for HPLC analyses.

**Conventional liquid solvent extraction.** Three different liquid solvents, water, ethanol and methanol, were used to extract asiaticoside and asiatic acid from *Centella asiatica*. Asiatic acid and asiaticoside were extracted by charging 3 g of *Centella asiatica* in a 250 ml round bottom flask (Pyrex, USA) with 100 ml of either water, ethanol, or methanol. The extraction was carried out at 25° C for 24 h or at the solvent boiling point for 5 h, while stirring at 1200 rpm using a magnetic stirrer. The mixture was filtered through a 0.4 µm Nylon membrane filter to obtain extracts. Each solvent

extraction was carried out in triplicate.



**Figure 1 :** Schematic diagram of subcritical water extraction system



**Figure 2 :** Flow chart of subcritical water extraction separation.

**Analysis.** High performance liquid chromatography (HPLC) was used for analysis of asiaticoside and asiatic acid. Four different schemes to measure extraction yields were performed in this study, as shown in Figure 2. Chromatographic separations were performed with a mobile phase of water and acetonitrile with a gradient of acetonitrile: 20-55% (30 min), 55% (5min), 55-20% (5 min) at a flow rate of 1.4 mL/min [8]. The amount of sample injection was set at 20 µL. The column was Optimapak C18 (25 cm x 4.6 mm, 5µm). The morphology of filtered particles was examined using scanning electron microscopy (SEM, Hitachi S-4200). The particle size in subcritical water extracts was measured using a dynamic light scattering (DLS) technique with ELS-8000 instrument (Photal Otsuka Eng., Japan).

## RESULTS AND DISCUSSION

### Conventional liquid solvent extraction

Table 1 lists the extraction yield of asiatic acid and asiaticoside using either water, ethanol or methanol. The extraction conditions were 25°C and 24 h or the boiling point of each solvent and 5 h. When water at 25° and 100°C was used, asiatic acid was not extracted and only a small amount of asiaticoside (1-1.3 mg/g) was extracted. When methanol at 25°C was used, the extraction yield of asiatic acid was 2.2 mg/g and the extraction yield of asiaticoside was 8.2 mg/g. When ethanol at 25°C was used, the extraction yield of asiatic acid was 2.0 mg/g and the extraction yield of asiaticoside was 5.2 mg/g. This may suggest that the polarity of solvent is responsible for the extraction yield of asiatic acid and asiaticoside. Extraction yield of asiatic acid and asiaticoside increased as the extraction temperature increased; when methanol was used at its boiling point (65°C), the extraction yield of asiatic acid was 2.3 mg/g and asiaticoside was 12.4 mg/g. The extraction yield of asiatic acid

was 10.0 mg/g and asiaticoside was 17.7 mg/g when ethanol at its boiling point (78°C) was used. Thus higher extraction temperature is beneficial to extract larger amount of asiatic acid and asiaticoside.

Table 1. The extraction yield of asiatic acid and asiaticoside using conventional liquid solvent extraction

	Water		Methanol		Ethanol	
	25°C	100°C	25°C	65°C	25°C	78°C
Asiatic acid (mg/g)	0	0	2.2	2.3	2.0	10.0
Asiaticoside (mg/g)	1.0	1.3	8.2	12.4	5.2	17.7

### Subcritical water extraction

Effects of temperature and pressure on asiatic acid and asiaticoside extraction yield were examined by varying temperature ranging 100-250 °C and pressure ranging 10-40 bar at 5 hr and the results are shown in Figures 3 and 4. The extraction yield of asiatic acid increased significantly from 0 to 7.0 mg/g and asiaticoside increased significantly from 1.1 to 8.4 mg/g when temperature increased from 100 to 250 °C. The enhanced solubility and the enhanced transport properties of subcritical water at higher temperature may be responsible for the higher extraction yields of asiatic acid and asiaticoside. The extraction yields are not strongly dependent on the extraction pressure. As pressure increased from 10 to 40 MPa, the extraction yield of asiaticoside slightly increased from 4.6 to 8.1 mg/g, and the extraction yield of asiatic acid slightly increased from 2.4 to 3.4 mg/g. The polarity of the subcritical water does not change much with pressure, as the result, the subcritical water pressure does not have a strong effect on the extraction yield.

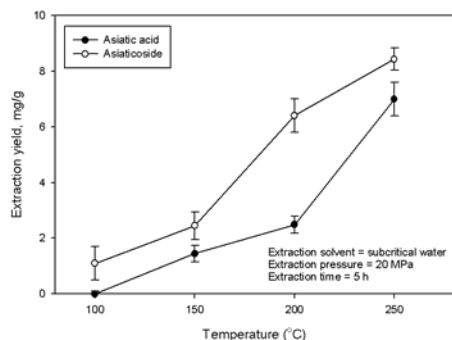


Figure 3 : Effect of temperature

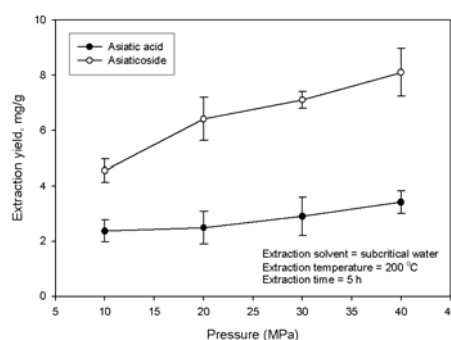


Figure 4 : Effect of pressure

Table 2 shows the extraction yield of asiatic acid, asiaticoside at an optimized condition (250 °C, 400 bar, 5 h), and the average particle sizes on the filters and filtrates. The extraction yield of asiatic acid was 7.8 mg/g and asiaticoside was 10.0 mg/g when the Direct analysis was used. The particle size of the extracts was measured to be 0.81 µm. When Separation scheme 1 was used, the extraction yield of asiatic acid present on the 8 µm size filter was 7.0 mg/g, which is close to the

extraction yield of the Direct analysis. In the filtrate solution penetrating through the 8  $\mu\text{m}$  size filter, no asiatic acid was detected while 8.4 mg/g of asiaticoside was present. The size of particles present on the 8  $\mu\text{m}$  size filter was larger (1.10  $\mu\text{m}$ ) than the size of particles present in the filtrate (0.68  $\mu\text{m}$ ). Thus, the larger particles containing most of the asiatic acid can be isolated from the other components in the extract. When Separation scheme 2 was used, no asiatic acid was present on the 0.4  $\mu\text{m}$  size filter while 7.3 mg/g asiatic acid was present. In the filtrate, no asiatic acid and no asiaticoside were detected. The size of the particles present on the 0.4  $\mu\text{m}$  size filter was larger (0.80  $\mu\text{m}$ ) than the size of particles present in the filtrate (0.40  $\mu\text{m}$ ). Thus, the particles containing most of asiaticoside can be isolated from the particles containing asiatic acid as well as the particles containing other components in the extract based on their particle size. When Separation scheme 3 was used, the extraction yield of asiatic acid (7.1 mg/g) and asiaticoside (9.3 mg/g) present on the 8  $\mu\text{m}$  size filter was very close to the extraction yields of the Direct analysis. In the filtrate, no asiatic acid or a very small amount of asiaticoside (0.1 mg/g) was present. The size of particles present on the 0.4  $\mu\text{m}$  size filter was larger (1.00  $\mu\text{m}$ ) while the size of particles present in the filtrate was smaller (0.43  $\mu\text{m}$ ) than that of the subcritical water extracts (0.81  $\mu\text{m}$ ). This indicates that the particles containing asiatic acid and the particles containing asiaticoside can be separated from the particles containing other components using the 0.4  $\mu\text{m}$  size filter.

Table 2. The extraction yield of asiatic acid and asiaticoside at an optimized conditions

		Asiatic acid (mg/g)	Asiaticoside (mg/g)	Particle size ( $\mu\text{m}$ )
Direct analysis		7.8	10.0	0.81
Separation scheme 1	Filter	7.0	-	1.10
	Filtrate	-	8.4	0.68
Separation scheme 2	Filter	-	7.3	0.80
	Filtrate	-	-	0.40
Separation scheme 3	Filter	7.1	9.3	1.00
	Filtrate	-	0.1	0.43

Figure 5 shows SEM images of extracted particles containing (a) asiatic acid on the 8  $\mu\text{m}$  size filter (Separation scheme 1), (b) asiaticoside on the 0.4  $\mu\text{m}$  size filter (Separation scheme 2), and (c) asiatic acid and asiaticoside on the 0.4  $\mu\text{m}$  size filter (Separation scheme 3). It can be seen that particles containing asiatic acid are larger than the particles containing asiaticoside. The diameter of the particles containing asiatic acid was measured to be 1.21  $\mu\text{m}$  with a particle size distribution of 1.31 and the diameter of asiaticoside particles was 0.76  $\mu\text{m}$  with relatively narrow particle size distribution (1.08). The size of particles containing asiatic acid and asiaticoside measured with SEM agree well with those measured with DLS. Due to the difference of size of particles containing asiatic acid and asiaticoside, asiatic acid and asiaticoside can be separated using the simple filtering process. When the subcritical extracts were filtered using the 0.4  $\mu\text{m}$  size filter, larger particles with

1.92  $\mu\text{m}$  in diameter and smaller particles with 0.58  $\mu\text{m}$  in diameter were observed (Figure 5c). This may indicate both of the particles containing asiatic acid and asiaticoside were collected using the 0.4  $\mu\text{m}$  size filter.

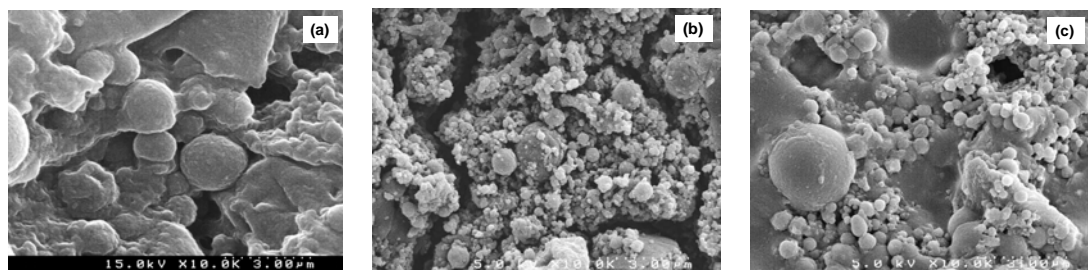


Figure 5 : SEM image of filtered particles produced by subcritical water extraction at 250 °C, 40 MPa, 5 hr on (a) the 8  $\mu\text{m}$  size filter (Separation scheme 1 ), (b) the 0.4  $\mu\text{m}$  size filter (Separation scheme 2), (c) the 0.4  $\mu\text{m}$  size filter (Separation scheme 3)

## CONCLUSION

In the extraction of *Centella asiatica* using subcritical water, the extraction yields of asiatic acid and asiaticoside increased with an increase in temperature, whereas the extraction yields were not markedly increased with an increase in pressure. The optimum extraction conditions of asiatic acid and asiaticoside were at 250 °C and 40 MPa. Under these conditions, 7.8 mg/g of asiatic acid and 10.0 mg/g of asiaticoside were extracted. The extraction yields of asiatic acid and asiaticoside, using subcritical water at optimum conditions, were higher to those using conventional liquid solvent extraction, using water and methanol, while its values were lower to those using a conventional liquid solvent extraction using ethanol. The extracted asiatic acid and asiaticoside were precipitated when the subcritical water containing the extracts were depressurized and cooled to room temperature. This enables the collection of asiatic acid and asiaticoside in particle form using the simple filtering process. The size of the particles containing asiatic acid (1.21  $\mu\text{m}$ ) was larger than the size of the particles containing asiaticoside (0.76  $\mu\text{m}$ ). Thus, separation of asiatic acid and asiaticoside can be achieved when choosing an appropriate size of filter.

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