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Extraction of bioactive components from *Centella asiatica* using subcritical water

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ABSTRACT

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 the optimal extraction conditions of 40 MPa and 250 °C, the extraction yield of asiatic acid was 7.8 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 (1.21 μm) than particles containing asiaticoside (0.76 μm). 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 methanol or ethanol at room temperature while the subcritical water extraction yields were lower than extraction yields with methanol or ethanol at its boiling point temperature.

1. Introduction

Bioactive natural products have an enormous economic importance as specialty chemicals. Bioactive natural products can be used as drugs, biological or pharmacological ingredients, nutraceuticals, and raw materials for the production of drugs [1]. *Centella asiatica* is a tropical medicinal plant with a long history of therapeutic uses for conditions such as dermal disorders, venous insufficiency, and microangiopathy [2]. Previous studies demonstrated that *C. asiatica* extracts can enhance collagen synthesis *in vitro* and extracellular matrix accumulation *in vivo* [3], and can enhance tensile strength in wound tissue and facilitate the wound healing process [4]. Four main bioactive compounds in *C. asiatica* are asiatic acid **(1)**, asiaticoside **(2)**, madecassic acid **(3)**, and madecassoside **(4)** as shown in Fig. 1 [5]. Among them, asiaticoside is the principal bioactive ingredient in *C. asiatica* since asiaticoside retains the most profound effect on antibacterial and fungicidal activity against pathogens and fungi [6]. Asiatic acid also exhibits bioactive efficacy [7]. For example, asiatic acid is known to control cell division in human hepatoma, colon cancer, breast cancer, melanoma cells and cytotoxic activity on fibroblast cells [8]. Thus, the synthesis and pharmacological mechanism of asiatic acid derivatives have drawn considerable interest.

Typically, bioactive compounds in herbal plants are present in low concentrations. Thus, it is very important to develop more effective and selective extraction methods for the recovery of the desired bioactive compounds from the herb materials. Traditional organic solvent-based extraction often suffers from low extraction yields, long extraction times, and residual toxic organic solvents in final products. The residual solvents are problematic because residual toxic organic solvents in extracts can deteriorate the quality of the extracts and can cause serious health problems when the extracts are taken into the human body. Hence, high level of vacuum under heating, that is a high energy-consuming evaporation process, is often needed to remove the residual solvents in the extracts to permitted levels. Supercritical fluid extraction (SFE), especially supercritical carbon dioxide ($scCO_2$) extraction, of bioactive materials from herb plants is a potential alternative to conventional liquid solvent extractions [9]. The major disadvantage of $scCO_2$ extraction, however, is that extraction of polar components is highly limited by the poor solvent power of $scCO_2$ for the polar components.

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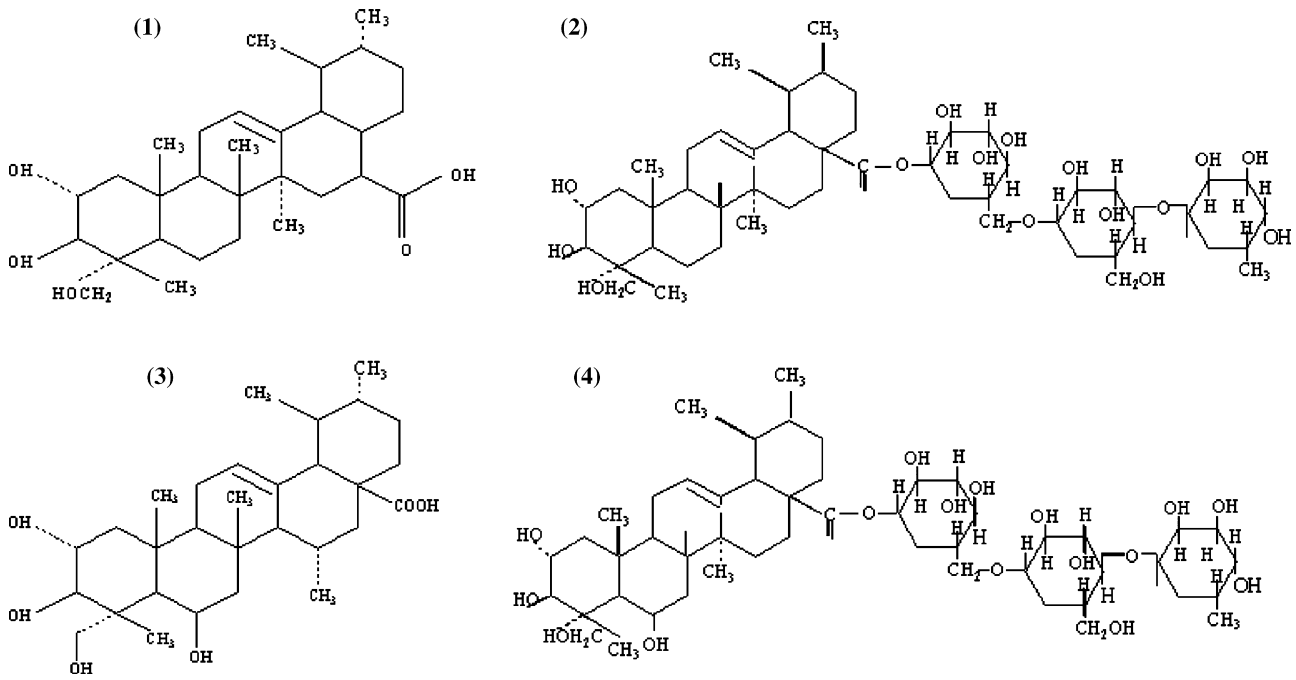

Fig. 1. Chemical structures of asiatic acid **(1)**, asiaticoside **(2)**, madecassic acid **(3)** and madecassoside **(4)** from Ref. [5].

Subcritical water extraction, using water under external pressurization above its boiling point as an extraction solvent, received much attention to extract desired polar compounds from herbs or plants [10]. Subcritical water extraction offers an efficient, non-toxic, and environmental-friendly alternative to conventional organic liquid solvent extraction techniques. Other important advantages of subcritical water extraction over the organic liquid solvent extraction include shorter extraction time, higher quality of extracts, and lower solvent extraction costs [11]. Subcritical water as an extraction solvent has been explored to extract polar, bioactive components from herbs and foods. It has been shown that 80% of oxygenates from savory and peppermint can be extracted with subcritical water at 6 MPa and 100 °C [12], 54% of nutraceuticals from oregano can be extracted at 10 MPa, 200 °C [13], and 90–95% of lignans from whole flaxseed can be extracted at 5.2 MPa and 140 °C [14].

In the past, organic solvents such as methanol [15], ethanol [16,17], or methanol–water mixture [18] were used to obtain extracts from *C. asiatica*. This study describes extraction of asiatic acid and asiaticoside from *C. asiatica* with subcritical water. 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.

2. Material and methods

2.1. Materials

Dried *C. asiatica* were obtained from Dexa Medica Pharmaceutical Company (Jakarta, Indonesia). The sample contains leaves, nodes, petioles and roots of *C. asiatica*. The *C. asiatica* were ground to an average particle size of 520 μm. Asiatic acid standard was purchased from Sigma Aldrich Co. (St. Louis, MO, USA) and asiaticoside standard (purity of 98.5%) was purchased from Fluka (Oakville, Ontario, Canada). Acetonitirle (HPLC grade) and methanol (HPLC grade) were obtained from J. T. Baker (Phillipsburg, NJ, USA) and ethanol (purity of 99.5%) was obtained from Junsei Chemical Co. (Tokyo, Japan). Deionized water was prepared using a Milli-Q, Ultrapure water purification system with a 0.22-μm filter (MA, USA). 8 μm filter papers, 0.4 μm Nylon membrane filters, and 0.4 μm polycarbonate membrane filters were purchased from Whatman (Maidstone, UK).

2.2. Extraction apparatus and procedure

The extraction experiments were conducted using a custom-built, subcritical water extraction apparatus. Fig. 2 shows a schematic diagram of the extraction apparatus. The extraction vessel (4) was made of SUS 316 with an internal diameter of 28 mm and a height of 377 mm, giving an internal volume of 232 ml. The preheater (3) was a 80 cm length SUS 316 coil with an internal diameter of 0.635 cm. The extraction vessel and the preheater were manufactured at the Korea Institute of Science and Technology machine shop. The temperature of the extraction vessel and the preheaters were controlled using a furnace (5, Daepoong Industries, Korea). The temperatures of the extraction vessel were monitored by inserting type-K thermocouples (model TJ36CAXL, Omega Engineering, Inc., USA) with a probe diameter of 0.16 cm inside the extraction vessel. The thermocouple has 0.3 s of response time with uncertainty measurement of 1 °C. The thermocouples were connected to a multichannel recorder (model DR 240, Yokogawa, Japan). The system temperature was controlled by a PID temperature controller (model DX 7, Hanyoung Industries, Korea). The high-pressure pump (2) was a model Pulsa 608 diaphragm metering pump, manufactured by Pulsa Feeder. Co. (NY, USA). This pump could generate pressures up to 50 MPa and could produce a maximum flow rate of about 1.2 l/h. The pressure of the extraction vessel was controlled using a back-pressure regulator (7), manufactured by Tescom (Model 44-2300 Series, MN, USA). The back-pressure regulator was rated to 100 MPa.

The procedure for extraction of asiatic acid and asiaticoside from *C. asiatica* consisted of several steps. *C. asiatica* (50 g) was charged into the extraction vessel. Deionized water was then introduced into the extractor using the high-pressure pump at an experimentally desired pressure. The pressure of the extractor was controlled