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Determination of Artemisinin Content in *Artemisia annua L.*

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Abstract

Artemisinin from dry leaves of *Artemisia annua L.* was extracted by supercritical fluid extraction using CO_2 as the solvent and Microwave-assisted extraction by different solvents as ethanol, chloroform, n-hexane and toluene. Extracts were analyzed in GC-MS system gas chromatograph, by comparison of the retention time with that of the standard artemisinin. Determination of arteannuin B, artemisinic acids were conducted by comparison of the spectrum with that of a library. The content of artemisinin was determined as 0.29-0.85% of dry weight for microwave-assisted extraction, and 0.32% for supercritical fluid extraction, for arteannuin B as 0.18-1.23% and 1.18%, for artemisinic acid as 0.32-2.33% and 0.66%, respectively. The yield of total extract obtained by supercritical fluid extraction was 1.70%, by microwave-assisted extraction - 1.33-8.62%. Best solvents for the artemisinin extraction were toluene, chloroform and ethanol.

Key words: artemisinin, supercritical extraction, arteannuin B, artemisinic acids

Introduction

Artemisia annua L. is the herb of the family Asteraceae, which known as Annual Wormwood is native in Asia. It has been used in Chinese traditional medicine for treatment of fever from ancient times. Artemisinin is naturally formed in *A. annua* and has mainly been detected in the aerial parts of the plant, particularly in the leaves, stems, buds and flowers. Its amount ranges from 0.01% to 1.54% (Charles & Simon, 1990; Ferreira *et al*., 1995) of dry weight according to various factors such as the plant's origin, stage of development and the cultivation condition. Artemisinin is a naturally occurring peroxidic sesquiterpene. Systematically, it is named [3R-(3a, 5ab, 6b, 9a, 12b, 1aR)]-octahydro-3, 6, 9-trimethyl-3, 12H-pyrano [4, 3-*j*]-1, 2-benzodioxepin-10(3H)-one (Avery *et al*., 2003).

The mechanism of action of artemisinin is still non conclusive. Experimental and theoretical studies suggest existence of several processes involving artemisinin, as forming carbon free radicals which alkylate specific malarial proteins causing lethal damage to parasites (Teja-Isavadharm *et al*., 2004), inhibition of heme polymerization by breaking hemozoin (crystalline heme aggregate, malaria pigment, hematin) (Ziegler *et al*., 2001) into heme units, or specifically inhibiting the parasite membrane Ca^{2+}-transporting ATP-ases (sarcoplasmic reticulum Ca^{2+}-transporting ATP-ase or SERCA) (Klayman *et al*., 1984). Artemisinin and its derivatives are the only group of compounds that is still effective against drug-resistant *P. falciparum* strains, and has the ability to quickly lower parasite level (Krishna *et al*., 2004).

Materials and Methods

Plant material. The plant sample of *A. annua* was collected in the vicinity of Ulaanbaatar city, Mongolia in August 2006, and air dried. The leaves were separated from the other parts of plants and for artemisinin extraction.

Supercritical fluid extraction. The supercritical fluid extraction (SFE) method uses CO_2 as a solvent. In comparison with the Soxhlet extraction, this method has many advantages such as lower solvent consumption and lower working temperature. This method offers other advantages related to the favorable properties of supercritical fluids: low viscosity, high solute diffusivity, improved mass transfer and reduction of extraction time. The appearance of the SFE extracts depends on the operational condition used during the extraction. Extraction process of

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