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Extraction time and temperature affect the extraction efficiencies of coumarin and phenylpropanoids from *Cinnamomum cassia* bark using a microwave-assisted extraction method

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ABSTRACT

Microwave-assisted extraction (MAE), an efficient extraction tool, was employed to extract a coumarin and five phenylpropanoids (cinnamic acid, cinnamyl alcohol, cinnamaldehyde, 2-hydroxycinnamadehyde, and 2-methoxycinnamaldehyde) from *Cinnamomum cassia* bark using water as the extraction solvent. Six marker compounds were quantified by high-performance liquid chromatography-diode array detector using a validated analytical method. To investigate the influences of temperature and time on the extraction yields of the six marker compounds, the water extracts of *C. cassia* bark were prepared using a MAE method at six different extraction temperatures (70, 75, 80, 85, 90, and 95 °C) and times (2, 4, 6, 8, 10, and 12 min). Their influences were assessed by multiple regression analysis. The results obtained demonstrated that higher extraction temperature and longer extraction time positively affected coumarin and cinnamyl alcohol contents, but negatively affected extract contents of cinnamic acid, cinnamaldehyde and 2-hydroxycinnamaldehyde (all $p- < 0.05$). The extraction of 2-methoxycinnamaldehyde was affected by both positively and negatively by increasing temperature and time. These changes during MAE were assumed by the chemical natures of the marker compounds with various functional groups. In conclusion, temperature and times significantly affected the extraction efficiencies of a coumarin and five phenylpropanoids from *C. cassia* bark when a water-based MAE method was used. This study provides a novel approach to the preparation of the water extract of *C. cassia* bark using MAE.

1. Introduction

The bark of *Cinnamomum cassia* (L.) J.Presl (Lauraceae) has been used to treat disorders such as cold intolerance, weakness, soreness and coldness of lower back and knees, to promote digestion, and to relieve spasmodic pain in the gastrointestinal tract [1]. Recent pharmacological researches report that the aqueous or ethanol extract of *C. cassia* bark exhibits neuro-protective effects on glutamate-induced neuronal death in cultured rat cerebellar granule cells, suppresses nitric oxide production in RAW 264.7 cells, and induces angiogenesis and has anticancer activity in human cervical cancer cell lines [2–5]. Bioactive compounds in *C. cassia* bark, such as, cinnamaldehyde, cinnamyl alcohol, cinnamic acid, 2-methoxycinnamaldehyde, and 2-hydroxycinnamaldehyde, have also been reported to suppress nitric oxide production, angiogenesis, and reduce rat myocardial ischemia/reperfusion injury, and platelet anti-aggregation activities [3,4,6,7]. Hence, various extraction methods have been developed to isolate the active compounds in *C. cassia* bark, for example, heating in water [2,5], macerating in methanol [3], and by using ultra-sonic extraction in methanol or aqueous methanol [8–11].

Microwave-assisted extraction (MAE), which is a combination of microwave and solvent extraction, has been employed to extract active compounds from plant materials [12], because as a extraction method, it can provide higher extraction efficiencies in shorter times at lower consumed solvent levels than conventional reflux methods [13,14]. These advantages of MAE are ascribed to the way that microwave energy is delivered to molecules. When microwave energy is delivered directly to solvents and herbal materials, the energy is converted into kinetic energy, which can promotes dipole rotation and ionic conduction [15,16]. Microwave energy creates thermal energy within a solvent and materials via a synergistic effect of heat and mass transfer working in the same direction [17,18]. These heating characteristics enable the MAE method to be used for the extraction of bioactive compounds from herbal medicines, for example, glycyrrhizin from *Glycyrrhiza uralensis* rhizomes and roots, puerarin from Puerariae Radix, ginsenosides Rg_1 and Rb_1 from *Panax ginseng* root, and astragalosides from *Astragalus membranaceus* var. *mongholicus* roots [19–22]. However, no previous report has been issued on the use of MAE to prepare the water extract of *C. cassia* bark.

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High-performance liquid chromatography-diode array detector has been widely adopted to quantify bioactive compounds in *C. cassia* bark, because it is simpler, faster, more reliable and cost efficient than liquid chromatography-mass spectrometry based methods for the analysis of coumarin and phenylpropanoid-related compounds, such as, cinnamic acid, cinnamaldehyde, cinnamyl alcohol, 2-methoxy cinnamaldehyde, and 2-hydroxycinnamaldehyde [8–11]. Therefore, it is thought that these phenylpropanoid-type compounds and coumarin can be employed as the marker compounds of *C. cassia* bark as these compounds are representative of the chemical make-up of *C. cassia* bark.

In the present study, the analytical methods used to quantify the six marker compounds in the water extract of *C. cassia* bark were established using validated HPLC-DAD methods. MAE was used to prepare the water extract of *C. cassia* bark using six different heating times at six different temperatures to investigate the influence of extraction time and temperature on the extraction yields of these six marker compounds in *C. cassia* bark. Multiple regression analysis was used to identify relationships between the extraction variables (extraction temperature and extraction time) on the yields of the marker compounds.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade solvents, acetonitrile and water, were purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA). Trifluoroacetic acid (TFA) was purchased from Sigma-Aldrich (St Louis, MO, USA). Coumarin and cinnamyl alcohol were purchased from Wako Pure Chemical Industries Ltd. (Osaka Japan). Cinnamic acid, cinnamaldehyde, and 2-methoxycinnamaldehyde were obtained from Sigma-Aldrich (St Louis, MO, USA). 2-Hydroxycinnamaldehyde was purchased from Tokyo Chemical Industry (Tokyo, Japan). All reagents and marker compounds had purities of ≥ 98%.

Dried *Cinnamomum cassia* bark was purchased from herbal medicine company (Kwangmyungdang Medicinal Herbs; Ulsan, Korea) and authenticated by the author (JH Kim). Voucher specimen (2017-PNUKM-CC-01) has been deposited at the School of Korean Medicine, Pusan National University.

2.2. Sample preparation

Crude drugs of *C. cassia* bark was pulverized and homogenized using a 500 μm testing sieve (Chunggye, Korea). Five hundred grams of powdered *C. cassia* bark was then extracted into 20 mL of distilled water (w/v) using a microwave oven (BP-111-RS; Microwave Research & Applications, Inc., IL, USA) for 2, 4, 6, 8, 10, and 12 min at 70, 75, 80, 85, 90, and 95 °C, and then the extract was centrifuged at 3000 rpm for 10 min. Supernatant was transferred to a 15 mL conical tube and lyophilized using a freeze-dryer (IlShinBioBase, Dongducheon, Gyeonggi-do) to produce powder. Accurately weighed lyophilized powder (5 mg) was dissolved in 1 mL of HPLC-grade water to a concentration of 5000 μg/mL. The solution was centrifuged at 10,000 rpm for 5 min and filtered through a 0.2 μm syringe filter (BioFact, Daejeon, Korea) prior to HPLC injection.

2.3. Chromatographic conditions

An Agilent 1260 liquid chromatography system (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto-sampler, degasser, quaternary solvent pump, and diode array detector was used for the quantitative analysis. Data were processed using Chemstation software (Agilent Technologies Inc., USA). The six marker compounds were separated on Eclipse C_{18} column (4.6 mm × 150 mm, 5 μm; Agilent Technologies) at 35 °C. The flow rate was 1 mL/min and the injection volume was 5 μL. The mobile phase consisted of solvent A (water containing 0.1% TFA) and solvent B (acetonitrile), and elution was conducted using the gradient elution program as follows: 20% (B) for 0–2 min, 20–50% (B) for 2–20 min, and held for 2 min. The column was then re-equilibrated with 20% (B) until the end of the analysis. The diode-array detector was set at ultraviolet wavelengths of 250, 280, and 295 nm.

2.4. Method validation

2.4.1. Calibration curves, linearity, limits of detection (LODs) and limits of quantification (LOQs)

Accurately weighed marker compounds were dissolved in methanol at a concentration of 1000 μg/mL to make stock solutions and then diluted to produce six working solutions of different concentrations. These working solutions injected to HPLC to construct calibration curves of absolute area of a compound peak versus compound concentration. The linearity of each calibration curve was evaluated using correlation coefficients (r^2). LOD and LOQ were determined as signal-to-noise (S/N) ratio of 3 and 10, respectively.

2.4.2. Precision

Precisions of the analytical methods were determined by analyzing low, middle, and high concentrations of the above-mentioned working

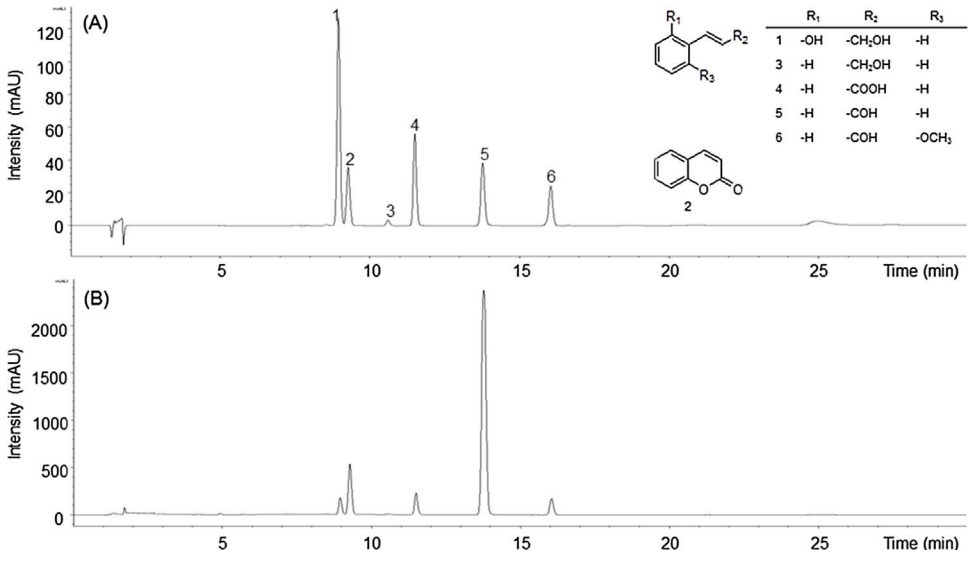

Fig. 1. Chromatograms of the six marker compounds (A) and of the water extract of *Cinnamomum cassia* bark (B) at a detection wavelength of 280 nm 1, 2-Hydroxycinnamaldehyde; 2, coumarin; 3, cinnamyl alcohol; 4, cinnamic acid; 5, cinnamaldehyde; 6, 2-methoxycinnamaldehyde.