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Determination of madecassoside and asiaticoside contents of *C. asiatica* leaf and *C. asiatica*-containing ointment and dentifrice by HPLC-coupled pulsed amperometric detection

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ABSTRACT

The present study reports the development and application of an HPLC-coupled pulsed amperometric detection method to determine the madecassoside and asiaticoside contents of *Centella asiatica* leaf and of commercial *C. asiatica*-containing ointment and dentifrice. *C. asiatica*, which was not pretreated, was extracted with 50% ethanol for 10 min. Madecassoside and asiaticoside were separated on a C18 column within 5 min using 25% (v/v) acetonitrile as the mobile phase. Both compounds were detected with high sensitivity when sodium hydroxide was used as a post-column eluent. Madecassoside and asiaticoside both displayed limits of detection of 0.005 μg/mL and linear regression coefficients of 0.9994 and 1.0000, respectively. The intra- and inter-day precisions were<8.85% and average recovery was>94.79%. The madecassoside and asiaticoside contents of ointment and dentifrice were successfully determined without sample purification or concentration owing to the high method sensitivity and selectivity.

1. Introduction

Centella asiatica (Apiaceae) is used worldwide to treat skin inflammation due to wounds, burns, and other causes. The main bioactive compounds of *C. asiatica* include triterpenoids such as madecassoside, asiaticoside, madecassic acid, and asiatic acid [1,2]. The chemical structures of the compounds are shown in Fig. 1. Several studies have reported the anti-inflammatory [3], antioxidant [4,5], cardioprotective [6,7], and neuroprotective effects [8–10] of these compounds. *C. asiatica* is widely used in natural medicines including ointments, dentifrices, and cosmetics. Recently, commercial dentifrice containing *C. asiatica* has been developed based on the study which showed an improvement of oral conditions from the extracts of *C. asiatica* [11]. According to the study, *C. asiatica* clearly suppressed the damage of gingival cell, accelerated the regeneration of gingiva, reduced the effects of dental plaque index and gingivitis index, and showed antibacterial effects [11]. Therefore, the analysis of bioactive compounds of *C. asiatica* in dentifrices is required to quality control the dentifrices. However, analysis of madecassoside and asiaticoside in dentifrices is very difficult due to the interference from other components such as polishing, foaming, flavouring agents, sweetener, humectant, thickening agents, and preservative. Moreover, for the analysis of these natural compounds in dentifrices, a high sensitive method is required because these compounds only exist in small amounts. As the release of these natural herbal dentifrices is increasing, establishment of a suitable analytical method for natural products has become an important matter. Therefore, a highly sensitive and selective analytical method which can be applied to the natural herbal commercial products (e.g., ointment and dentifrice) is needed.

Although the triterpenoids of *C. asiatica* are pharmaceutically important, few analytical methods for these compounds have been reported. Thin-layer chromatography-mass spectrometry has proven to be insufficiently accurate [12]. The madecassoside, asiaticoside, and their aglycones were successfully determined in *C. asiatica* using HPLC-UV method [13,14]. However, triterpenoid compounds are generally detected with low sensitivity at only short wavelengths (< 210 nm) because of their poor chromophoric properties. HPLC-ELSD is an alternative method when glycosides are difficult to determine by UV, but shows insufficient sensitivity for dentifrices [15].

Pulsed amperometric detection (PAD) utilizes a flowing current to initiate chemical conversion of the electro-active analytes that undergo redox reactions [16]. This technique allows the highly specific and highly sensitive (ppb) detection of carbohydrates [17]. Sample preparation with PAD is simple because non-sugar compounds do not interfere and clean-up procedures are typically not required [16]. High-performance anion-exchange chromatography (HPAEC)-PAD is frequently used to quantify carbohydrates in plant

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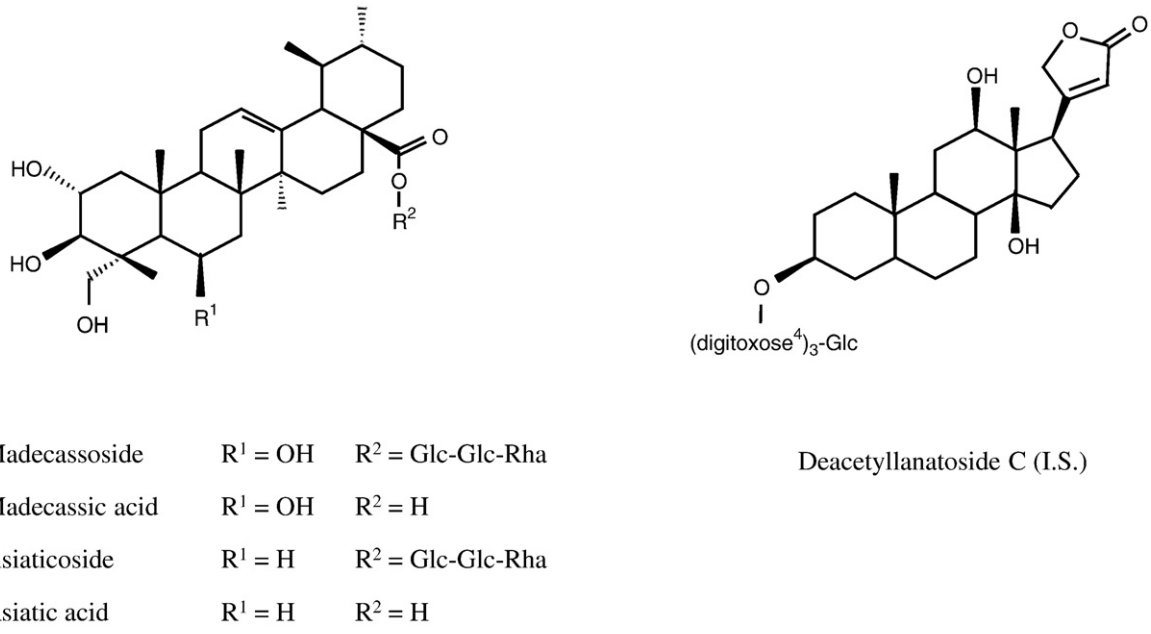

Fig. 1. Chemical structures of madecassoside, asiaticoside, and deacetyllanatoside C (as I.S.).

resources [18–22] because of its strong anion-exchange properties that efficiently separate carbohydrates. It allows the direct detection of carbohydrates with high sensitivity (about ng levels) [20]. Because of their sugar moieties, glycosides have previously been analyzed using reversed-phase HPLC-PAD [23], first separating the compounds by chromatography followed by detection. Sodium hydroxide solution is added to post-column reagents because sugar is converted to anions, which can be detected by PAD under strongly alkaline conditions. The reversed-phase HPLC-PAD method used to analyze herbal medicines, but applications of ointment or dentifrices were not yet reported.

The present study reports the development of a highly sensitive PAD-based method for determining the madecassoside and asiaticoside contents of *C. asiatica* and related commercial products (e.g., ointment and dentifrice). This method incorporates a simple pretreatment process that does not require sample purification or concentration. The limits of detection (LODs) and of quantitation (LOQs) by PAD were compared to those reported for UV and ELSD methods. Method precision and accuracy were evaluated by intra- and inter-day validations and recovery tests, respectively.

2. Experimental

2.1. Reagents and chemicals

Madecassoside, asiaticoside, and deacetyllanatoside C (as an internal standard, I.S.) were purchased from ChromaDex (Santa Ana, CA, USA). HPLC-grade acetonitrile and 50% sodium hydroxide were from Fisher Scientific (Fairlawn, NJ, USA). All other reagents and solvents used were of guaranteed or analytical grade. A membrane filter (Millipore type HA, pore size 0.45 μm) was used for solvent filtration. All samples were filtered through disposable syringe filters (Hydrophobic PTFE, pore size 0.20 μm, Advantec MFS, Tokyo, Japan) before injection. To prepare the standard, sample, and mobile phase solutions, 18 MΩ purified water (Automatic Aquarius AW-1001, Top Trading, Seoul, South Korea) was used. The weight of each sample was measured on a Mettler Toledo AX 105 DeltaRange (Greifensee, Switzerland). Amberlite XAD-2 was purchased from Sigma (St. Louis, MO, USA). *C. asiatica* leaf, ointment, and dentifrice were purchased from local stores.

2.2. Apparatus

HPLC equipment was purchased from Shiseido (Tokyo, Japan) and included a Model Nanospace SI-2/3201 pump, 3002 UV detector and 3004 column oven. The PAD system (ICS-3000 series, Dionex, Sunnyvale, CA, USA) was equipped with an Au-Flow cell containing a gold working electrode and a solvent-compatible cell containing an Ag/AgCl reference electrode. Chromatographic separation was performed using a Kinetex C18 column (100 × 2.1 mm I.D.; 2.6 μm, Phenomenex, Torrance, CA, USA). The potential waveform was as follows: E1 = −0.2 V (from 0.00 to 0.04 s); E2 = 0 V (from 0.05 to 0.21 s); E3 = +0.22 V (from 0.22 to 0.46 s); E4 = 0 V (from 0.47 to 0.56 s); E5 = −2 V (from 0.57 to 0.58 s); and E6 = +0.6 V (0.59 s). The following column parameters were used: mobile phase, isocratic elution of 25% (v/v) acetonitrile; flow rate, 0.2 mL/min; separation temperature, 30 °C; and injection volume, 10 μL. A post-column delivery system of 200 mM sodium hydroxide (flow rate: 0.8 mL/min) was added to the RP-HPLC-PAD system. The mobile phase was daily degassed through the system by vacuum filtration after a mixture of water and acetonitrile, followed by sonication for 20 min before use. Data were controlled on a computer running the Chromeleon client program (Dionex). The UV detector, which was set at 203 nm was controlled by a computer running the Dschromn program supplied by Donam Instrument (Seoul, South Korea).

2.3. Optimization of extraction efficiency

The optimal extraction conditions of madecassoside and asiaticoside in *C. asiatica* were investigated. Powder of *C. asiatica* leaf (1 g) was extracted under sonication for 30 min with 100 mL of 0–100% (v/v) ethanol solutions. The extracted solution was diluted with water and mixed with I.S. The final concentration of *C. asiatica* powder was 1 mg/mL, including 10 μg/mL I.S. Each sample was filtered through a disposable syringe filter before being quantified by HPLC. The best solvent was selected and 1 g of *C. asiatica* leaf powder was extracted