Determination of 5-Hydroxymethyl-2-furfural, Albiflorin, Paeoniflorin, Liquiritin, Ferulic Acid, Nodakenin, and Glycyrrhizin by HPLC-PDA, and Evaluation of the Cytotoxicity of Palmul-tang, a Traditional Korean Herbal Medicine

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INTRODUCTION

Many herbal formulae have been used to prevent and treat various diseases. Such herbal medicines have few side effects and exhibit multiple activities (Zhang et al., 2004; Jiang, 2005; Liu et al., 2008).

Palmul-tang (PMT) is a traditional herbal medicine that contains eight herbs, Ginseng Radix Alba, Glycyrrhizae Radix, Hoelen, Atractylodis Rhizoma, Angelicae Gigantis Radix, Cnidii Rhizoma, Paeoniae Radix, and Rehmanniae Radix Preparata. PMT has been employed in the treatment of qi deficiency and blood problems caused by consumptive disease (Hur, 2007). It has been reported to show antitumor (Ha et al., 2005), immunomodulatory (Kim, 1987; Ha and Nam, 1995), antifatigue (Ha and Baik, 2000), and antiallergic effects (Heo et al., 2003). However, no previous study has used high-performance liquid chromatography (HPLC) to determine the active components in PMT. Therefore, we simultaneously measured all such components for quality control purposes, and evaluated cytotoxicity of PMT extract.

HPLC coupled with photodiode array (PDA) detection is a convenient, widely used, and powerful approach for the rapid separation and identification of multiple components in herbal extracts and plants important in traditional Chinese medicine (Zhang et al., 2004; Park et al., 2009).

In the present study, we focused on quantitative determination of seven components of PMT, and used...
HPLC-PDA to this end. The components of interest were 5-hydroxymethyl-2-furaldehyde (1), albiflorin (2), paoniflorin (3), liquiritin (4), ferulic acid (5), nodakenin (6), and glycyrrhizin (7) (Fig. 1). Further, we investigated the cytotoxicity of PMT on the RBL-1 and BEAS-2B cell lines as well as splenocytes.

MATERIALS AND METHODS

**Chromatographic system**

A Shimadzu LC-20A HPLC system (Shimadzu Co.), consisting of a solvent delivery unit, an on-line degasser, a column oven, an autosampler, and a PDA detector, was employed. The data processor used LC-solution software (Version 1.24). A Gemini C18 analytical column (250 × 4.6 mm; particle size 5 µm; Phenomenex) was used. The mobile phases were solvent A (1.0% v/v aqueous acetic acid) and solvent B (1.0% v/v acetic acid in acetonitrile). The gradient flow was as follows: (A)/(B) = 95/5 (0 min) → (A)/(B) = 40/60 (40 min) → (A)/(B) = 0/100 (45 min; hold for 5 min) → (A)/(B) = 95/5 (55 min). Column temperature was maintained at 40°C. Analysis was performed at a flow-rate of 1.0 mL/min with detection wavelengths of 230 nm (2 and 3), 254 nm (7), 280 nm (1 and 4), 320 nm (5), and 330 nm (6). Each injection volume was 10 µL.

**Reagents and materials**

The compounds 1 and 5 were purchased from Sigma-Aldrich. 2, 3, and 7 were the products of Wako. 4 and 6 were purchased from NPC BioTechnology Inc. The purity of all reference standards was more than 98.0%. HPLC-grade methanol, acetonitrile, and water were obtained from J.T. Baker. Glacial acetic acid was of analytical reagent grade, procured from Junsei. The materials forming PMT were purchased from Omniherb and HMAX. A voucher specimen (2008-KE05-1~KE05-8) has been deposited at the Herbal Medicine EBM Research Center, Korea Institute of Oriental Medicine.

**Preparation of standard solutions and calibration curves**

Standard stock solutions of compounds 1-7 (all at 1,000 µg/mL) were prepared in methanol and stored below 4°C. Working standard solutions were prepared by serial dilution of stock solutions with methanol. All calibration curves were obtained by assessment of peak areas from standard solutions in the concentration ranges: 1, 6.25-50.00 µg/mL; 2, 5, and 7, 3.13-25.00 µg/mL; 3, 9.38-150.00 µg/mL; 4, 12.50-100.00 µg/mL; and 6, 4.69-75.00 µg/mL.

**Preparation of sample solutions**

A decoction of PMT was prepared in our laboratory (Table I) from a mixture of chopped crude herbs, extracted in distilled water at 100°C for 2 h. The solution was evaporated to dryness and freeze-dried (yield: 25.9%). Lyophilized PMT extract (200 mg) was dissolved in distilled water (20 mL) and mixed. The solution was filtered through a SmartPor GHP syringe filter (0.2 µm pore size, Woongki Science).

**Limits of detection (LOD) and quantification (LOQ)**

Stock solutions of reference compounds were diluted with methanol to assess LOD and LOQ values. The LOD and LOQ data obtained under the chromatographic conditions used in the present study were determined using signal-to-noise (S/N) ratios of 3 and 10.