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Original Article

Simultaneous determination of nine bioactive compounds in Yijin-tang via high-performance liquid chromatography and liquid chromatography-electrospray ionization-mass spectrometry

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ABSTRACT

Background: Yijin-tang (YJ) has been used traditionally for the treatment of cardiovascular conditions, nausea, vomiting, gastroduodenal ulcers, and chronic gastritis. In this study, a simple and sensitive high-performance liquid chromatography (HPLC) method was developed for the quantitation of nine bioactive compounds in YJ: homogentisic acid, liquiritin, naringin, hesperidin, neohesperidin, liquiritigenin, glycyrrhizin, 6-gingerol, and pachymic acid.

Methods: Chromatographic separation of the analytes was achieved on an RS Tech C_{18} column (4.6 mm × 250 mm, 5 μm) using a mobile phase composed of water containing 0.1% (v/v) trifluoroacetic acid (TFA) and acetonitrile with a gradient elution at a flow rate of 1.0 mL/min.

Results: Calibration curves for all analytes showed good linearity ($R^2 \geq 0.9995$). Lower limits of detection and lower limits of quantification were in the ranges of 0.03–0.17 μg/mL and 0.09–0.43 μg/mL, respectively. Relative standard deviations (RSDs; %) for intra- and interday assays were < 3%. The recovery of components ranged from 98.09% to 103.78%, with RSDs (%) values ranging from 0.10% to 2.59%.

Conclusion: This validated HPLC method was applied to qualitative and quantitative analyses of nine bioactive compounds in YJ and fermented YJ, and may be a useful tool for the quality control of YJ.

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1. Introduction

Yijin-tang (YJ, *Erchen-tang* in Chinese, *Nichin-to* in Japanese) is a traditional prescription for the prevention and treatment of diverse diseases; it has been used widely to treat nausea, vomiting, gastroduodenal ulcers, and chronic gastritis.[1] YJ has also been shown to improve digestive function in clinical research trials. Recently, YJ was reported to show therapeutic effects on diseases of the circulatory system and protective effects against gastric mucosa in in vivo experiments.[2,3] YJ is composed of *Pinellia ternata* Breitenbach, *Citrus unshiu* Markovich, *Poria cocos* Wolf, *Glycyrrhiza uralensis* Fisch, and *Zingiber officinale* Roscoe, according to the Korean Herbal Pharmacopeia. The major components of each herbal medicine in YJ are known to be bioactive components, including flavonoids (e.g., liquiritin, liquiritigenin, hesperidin, rutin, naringin, neohesperidin, and poncirin), triterpenoids (e.g., glycyrrhizin, pachymic acid, and eburicoic acid), phenolic acids (e.g., homogentisic acid), and pungent principles (e.g., 6-gingerol and 6-shogaol).[4–10] These compounds, derived from each herbal medicine, have various pharmacological activities including anticancer, -inflammatory, -bacterial, and -oxidant activities.[4,11–16] In this study, specific biomarkers were selected to evaluate the quality of YJ and its ingredients, including homogentisic acid for *P. ternata* Breitenbach, hesperidin, naringin and neohesperidin for *C. unshiu* Markovich, pachymic acid for *P. cocos* Wolf, liquiritigenin and glycyrrhizin for *G. uralensis* Fisch, and 6-gigerol for *Z. officinale* Roscoe (Table 1).

Several analytical methods for these compounds have been developed for qualitative and quantitative analyses using high-performance liquid chromatography-diode-array detector (HPLC-DAD) and liquid chromatography/mass spectrometry (LC/MS).[17–25] However, these methods cannot simultaneously determine the multiple bioactive components in YJ. Although a HPLC-DAD method to detect six compounds in YJ was recently developed, the identification and quantification of compounds based on retention times and UV spectra are limited due to the interference of nontarget molecular components in YJ.[26] Therefore, methods for simultaneously detecting these biomarkers in YJ is needed to ensure efficient quality control and pharmaceutical evaluation using LC/MS.

In this study, a rapid, simple, and efficient analytical method for nine biomarkers in YJ was developed using HPLC-DAD for quantitation and LC/MS for identification. Subsequently, the method was applied to chemically profile targeted analytes in YJ and *Lactobacillus*-fermented YJ.

Table 1 – Composition and biomarkers of five herbal medicines in Yijin-tang

Herbal medicine	Amount (g)	Ratio	Biomarkers
Pinellia ternata Breitenbach	800	41.0	Homogentisic acid
Citrus unshiu Markovich	400	20.5	Hesperidin, naringin, neohesperidin
Poria cocos Wolf	400	20.5	Pachymic acid
Glycyrrhiza uralensis Fisch	200	10.3	Liquiritigenin, glycyrrhizin
Zingiber officinale Roscoe	150	7.7	6-Gingerol

2. Materials and methods

2.1. Chemicals and reagents

Homogentisic acid, liquiritin, naringin, neohesperidin, and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA.). Liquiritigenin, 6-gingerol, and pachymic acid were obtained from Faces Biochemical Co., Ltd. (Wuhan, China). Hesperidin and glycyrrhizin were purchased from ICN Biomedicals (Santa Ana, CA, USA) and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), respectively. The purity of all standards was > 97%. All herbal medicines were purchased at the Yeongcheon traditional herbal market (Yeongcheon, South Korea). The chemical structures of the nine bioactive compounds are shown in Fig. 1. HPLC-grade acetonitrile was purchased from J.T. Baker Inc. (Philipsburg, NJ, USA). Deionized water was prepared using an ultrapure water production apparatus (Millipore, Billerica, MA, USA).

2.2. Preparation and fermentation of YJ

All herb samples were purchased from the Yeongcheon Herbal Store (Yeongcheon, South Korea) and all specimens (#50 for *P. ternata* Breitenbach, #22 for *C. unshiu* Markovich, #61 for *P. cocos* Wolf, #3 for *G. uralensis* Fisch, and #6 for *Z. officinale* Roscoe) were stored in the herbarium of the KM Application Center, Korea Institute of Oriental Medicine. Five medicinal herbs (1.95 kg, Table 1) were extracted in 19.5 L of distilled water for 3 hours using a COSMOS-660 extractor (Kyungseo Machine Co., Incheon, Korea). Extracts were sieved (106 μm) to yield 15.8 L of the decoction. To ferment YJ, 10 bacterial strains (including *Lactobacillus rhamnosus* KFRI 144, *Lactobacillus acidophilus* KFRI 150, *Lactobacillus amylophilus* KFRI 161, *L. acidophilus* KFRI 162, *Lactobacillus plantarum* KFRI 166, *L. acidophilus* KFRI 217, *Lactobacillus brevis* KFRI 221, *L. brevis* KFRI 227, *Lactobacillus curvatus* KFRI 231, and *L. acidophilus* KFRI 341) were obtained from the Korean Food Research Institute (Seongnam, Korea). Bacterial strains were cultured in de Man, Rogsa and Sharpe (MRS) broth at 37 °C for 24 hours and viable cell counts of each strain were determined in triplicate using a pour plate method on MRS agar. Solutions of YJ extracts were adjusted to pH 8 using 1 M sodium hydroxide and autoclaved for 15 minutes at 121 °C. Sterilized-YJ solutions (500 mL) were inoculated with 5 mL of the inoculum (1% v/v, 2×10^9 CFU/mL). Inoculated samples were incubated at 37 °C for 48 hours and a brownish powder of fermented YJ extract was obtained via lyophilization. These fermented YJ (f-YJ) samples were stored at 4 °C before use.

2.3. Preparation of calibration standards, quality control, and analytical samples

Standard stock solutions of the nine bioactive standards—homogentisic acid, liquiritin, naringin, hesperidin, neohesperidin, liquiritigenin, glycyrrhizin, 6-gingerol, and pachymic acid—were prepared at concentrations of 200 μg/mL,