Analysis of Constituents from Guibi-Tang with *Lactobacillus*

C. Liang, K. J. Lee, S. W. Jeong, J. H. Ha, and J. Y. Ma

***Abstract*—Guibi-tang (GB) is a traditional medicine used for anti-oxidant, anti-osteoporosis, hemostasis and gastroprotection. We investigated the levels of several compounds in GB before and after fermented with *Lactobacillus curvatus* KFRI 166 (GB166) and *Lactobacillus plantarum* KFRI 442 (GB442). The characterization of seven compounds were achieved by comparing the HPLC retention times (t_R) and UV absorptions of target peaks in the GB with those of standards liquiritin (1), nodakenin (2), nodakenitin (3), decursinol (4), 6-gingerol (5), decursinol angelate (6) and decursin (7). We are special ingredients of GB166 and GB442 were compared. As a result, the five compo-unds with the GB166 was decreased by *L. curvatus* KFRI 166, and the other nodakenin (2) and decursinol (4) were fermentation selective increased. But, GB442 was all compounds decreased. GB fermented with *Lactobacillus* affects the content of several compounds will be improves its absorption for develop the bioactivity.**

***Index Terms*—Guibi-tang, fermented, analysis, HPLC**

I. Introduction

Many traditional herbal medicines are used for the prevention and therapeutic treatment of diseases [1]. Indeed, many herbal therapies have become increasingly popular among patients and physicians, and they are now used by approximately 20% of the population in the United States because herbal medicines generally have few side effects and they are very effective [2]. However, concerns have been raised over the lack of quality control and scientific evidence for the safety of herbal medicine [3]. Besides, few scientific studies have explored the safety and toxicity of herbal medicines, so severalwa-rnings have been issued regarding the potential adverse effects of herbal medicines [4], [5].

Guibi-tang (GB) is a multi-herbal traditional Korean medicine that has been used for several hundred years to treat amnesia, poor memory or forgetfulness, fatigue, insomnia, anemia, palpitations, and neurosis. GB is composed of 12 herbs: *Angelica gigas* Nakai, *Dimocarpus longan* Lour, *Zizyphus jujuba* Miller, *Polygala tenuifolia* Willdenow, *Panax ginseng* C. A. Meyer, *Astragallus membranaceus* Bunge, *Atractylodes macrocephala* Koidzumi, *Pachyma hoelen* Rumph, *Aucklandia lappa* Decne, *Poria cocos* Wolf, *Glycyrrhiza uralensis* Fischer, and *Zingiber officinale* Roscoe. Bioconversion such as fermentation can maximize absorption of the active components from herbs as well as increase their bioactivity. Research on the effect of fermented with microorganisms on the quality and efficacy of medicinal herbs was conducted recently [6]-[8].

In this study, we fermented GB with *Lactobacillus*, which is widely used as a food material. *Lactobaci-llus* is known to inhibit the growth of some harmful bacteria by the production of lactic acid, and it has therapeutic effects, including anti-inflammatory and anti-cancer activities. To determine the changes of compounds in GB after fermentation, seven marker compounds, liquiritin (*Glycyrrhiza uralensis* Fischer), nodakenin (*Angelica gigas* Nakai), nodakenitin (*Ang-elica gigas* Nakai), decursinol (*Angelica gigas* Nakai), 6-gingerol (*Zingiber officinale* Roscoe), decursinol angelate (*Angelica gigas* Nakai) and decursin (*Angelica gigas* Nakai) were investingated. Amounts of the seven marker compounds in GB and fermented with *Lactobacillus curvatus* KFRI 166 (GB166) and *Lactobacillus plantarum* KFRI 442 (GB442) were measured by an established HPLC-DAD method.

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II. Experimental

A. Materials and Reagents

Samples of GB, GB166 and GB442 powder (3.0 g) were obtained from the Korea Institute of Oriental Medicine. HPLC grade solvents (water and acetone-trile) were purchased from J. T. Baker (USA). The compounds decursin, decursinol angelate, nodakenin, nodakenitin and 6-gingerol were purchased from the Korea Food & Drug Administration. Liquiritin was purchased from Wako (Japan), and decursinol was purchased from Sigma-Aldrich (USA). The purities of the seven standard compounds were greater than 98%.

B. Fermentation of Guibi-Tang and Preparation of Samples

The bacterial strain, *Lactobacillus curvatus* KFRI 166 and *Lactobacillus plantarum* KFRI 442 were obtained from the Korea Food Research Institute (KFRI, Korea). The test organism was transferred into MRS broth for *Lactobacillus* spp. and grown at 37 ℃ for 24 h. The activated culture was then inoculated into the broth under the same conditions. The culture was diluted to obtain an initial population of 1 – 5 × 107 CFU/ml and was designated as the inoculum. A GB water extract was used as the culture media for fermentation after adjusting the pH to 7.0 using 1 M NaOH and autoclaving for 15 min at 121 ℃. After cooling, 750 ml of GB was combined with 7.5 ml of the *Lactobacillus* inoculum described above. This was incubated at 37 ℃ for48 h. A powder of the GB166 and GB442 culture were prepared by freeze-drying. Powders

of GB, GB166 and GB442 (50 mg) were weighed accurately and dissolved in 1 ml of water. The samples were stored at 4℃ and filtered through a 0.45 μm membrane filter before analysis by HPLC

C. Analysis of Compounds in GB and FGB

HPLC system was an Elite Lachrom HPLC system (Hitachi High-Technologies Co., Tokyo, Japan) equipped with a pump (L-2130), an auto sampler (L-2200), a column oven (L-2350) and a diode array UV/VIS detector (L-2455). System control and data analyses were executed by EZchrom Elite software (version 3.3.1a). The analysis of compounds in the GB and FGB samples was conducted using a HECTOR C18 column (5 μm, 4.60 × 250 mm) at 40 ℃. The mobile phase consisted of acetonitrile (A) and water (B) at a flow rate of 1 ml/min. The mobile phase was a gradient of solvent A and solvent B as follows; 0 – 10 min, 1% A; 10 – 70 min, 50% A; 70 – 80 min, 50 – 100% A; 80 – 90 min, 100% A. The DAD detector UV wavelength was set at 203 nm according to the maximal UV absorption of seven compounds: liquiritin, nodakenin, nodakenitin, decursinol, 6-gingerol, decursinol angelate and decursin. The sample injection volume was 20 μl.

III. Result and Discussion

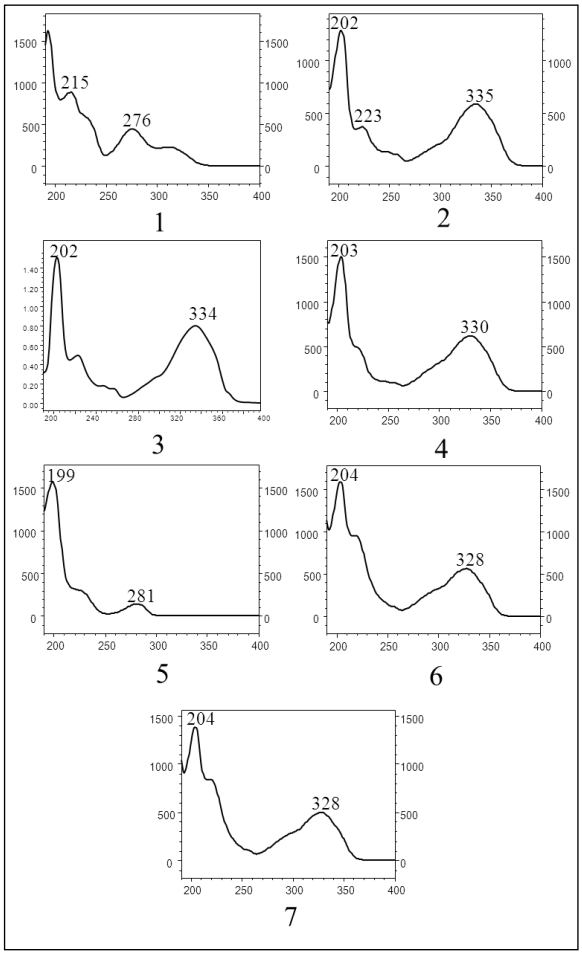

Fig. 1. UV spectrum of the seven compounds. (1. liquiritin, 2. nodakenin, 3. nodakenitin, 4. decursinol, 5. 6-gingerol, 6. decursinol angelate, 7. decursin).

We investigated the levels of several compounds in GB before and after fermented with *Lactobacillus curvatus* KFRI 166 (GB166) and *Lactobacillus plantarum* KFRI 442 (GB442). Total seven compo-unds were detected in GB, GB166 and GB442 by an established HPLC-DAD method. The characteriza-tion of seven compounds were achieved by comparing the HPLC retention times (t_R) and UV absorptions (Fig. 1) of target peaks in the GB with those of standards liquiritin (1), nodakenin (2), nodakenitin (3), decursinol (4), 6-gingerol (5), decursinol angelate (6) and decursin (7) (Fig. 2).

In GB 166, liquiritin (1), nodakenitin (3), 6-gingerol (5), decursinol angelate (6) and decursin (7) were decreased by 31.8 %, 78.4 %, 23.3 %, 55.7 % and 57.3 %, nodakenin (2) and decursinol (4) were increased by 0.9 % and 2.1 %, respectively, compared with GB. In GB442, liquiritin (1), nodakenin (2), nodakenitin (3), decursinol (4), 6-gingerol (5), decursinol angelate (6) and decursin (7) were both decreased by 14.9 %, 11.5 %, 6.6 %, 11.8 %, 11.0 %, 63.3 % and 63.8 %, respectively, compared with GB (Fig. 3). The nodakenitin is an aglycon of nodakenin, the decursinol is metabolism of the decursinol angelate and decursin. The nodakenitin, decursinol angelate and decursin was decreased, the nodakenin and decursinol was increased in GB166. So the *Lactobacillus curvatus* KFRI 166 may be don't metabolize the nodakenin to nodakenitin, only metabolize the decursinol angelate and decursin to decursinol.

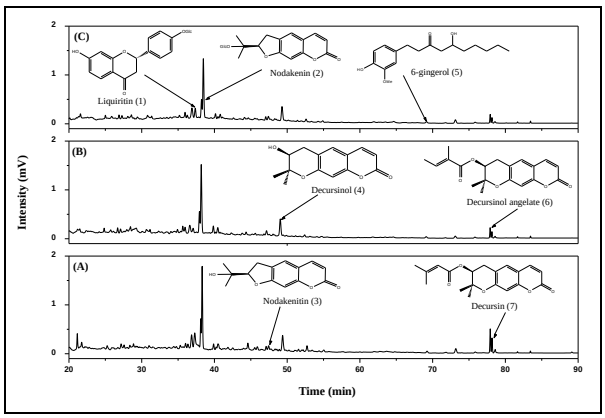

Fig. 2. HPLC chromatograms of GB (A), GB166 (B) and GB442 (C) at 203 nm with the structure of seven compounds.

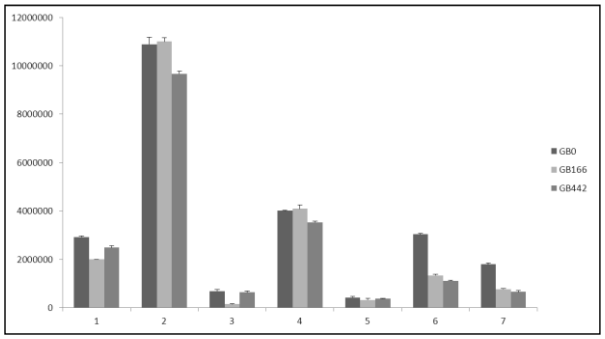

Fig. 3. Comparison amount of useful compounds in GB, GB166 and GB442. (1. liquiritin, 2. no-dakenin, 3. nodakenintin, 4. decursinol, 5. 6-gingerol, 6. decursinol angelate, 7. decursin).

IV. Conclusion

Total seven compounds were detected in GB, GB166 and GB442 by comparing the HPLC retention times (t_R) and UV absorptions of target peaks in the GB with those of standards.

We investigated the alteration of seven compounds in GB, GB166 and GB442. We are special ingredients of GB 166 and GB442 were compared. In conclusion, the five compounds with the GB166 was decreased by *L. curvatus* KFRI strain 166, and the other nodakenin (2) and decursinol (4) were fermentation selective increased. But, GB442 was all compounds decreased. GB fermented with *Lactobacillus* affects the content of several compounds will be improves its absorption for develop the bioactivity.

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