

Analysis of the Components of Guibitang and Fermented Guibi-tang and their Ability to Inhibit Angiotensin-converting Enzyme

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Abstract – Guibi-tang is a traditional medicine used for the treatment of colds. We investigated the levels of several compounds in Guibi-tang before and after fermentation with *Lactobacillus* and tested their ability to inhibit angiotensin-converting enzyme. Six known compounds (decursin, decursinol angelate, nodakenin, liquiritin, formononetin, and 6-gingerol) and 2 unidentified compounds were detected in Guibi-tang (GB) and fermented Guibi-tang (FGB) by an established HPLC-DAD method. The levels of the 6 known compounds were decreased after fermentation. FGB showed more potent inhibition of angiotensin-converting enzyme activity than GB. In conclusion, fermentation with *Lactobacillus* affects the content of several compounds in GB and improves its angiotensin-converting enzyme inhibitory activity.

Keywords – Guibi-tang, fermentation, angiotensin-converting enzyme

Introduction

Hypertension is a multifactorial process and is a risk factor or complication in many diseases, including cardiovascular disease, renal disease, and diabetes. Angiotensin-converting enzyme (ACE; peptidyldipeptide hydrolase EC 3.4.15.1) is a zinc-containing enzyme that plays an important physiological role in regulating blood pressure. This enzyme increases blood pressure by hydrolyzing the decapeptide angiotensin I to angiotensin II. The latter is a potent vasoconstrictor that stimulates the secretion of aldosterone. In turn, aldosterone promotes sodium and water retention in the kidneys and thus increases arterial pressure. ACE also catalyzes the degradation of the vasodilator bradykinin, further contributing to high blood pressure (Erdos, 1975; Hernandez-Ledesma *et al*, 2003; Skeggs *et al*, 1956). Thus, inhibition of ACE activity has an overall anti-hypertensive effect.

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, *Astragalus*

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 fermentation with microorganisms on the quality and efficacy of medicinal herbs was conducted recently (Kim *et al*, 2009; Doh *et al*, 2010; Hyon *et al*, 2009).

In this study, we fermented GB with *Lactobacillus*, which is widely used as a food material. *Lactobacillus* 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 (Chen *et al*, 2009; Goldin, 1998). To determine the changes in levels of compounds in Guibi-tang after fermentation, 6 marker compounds, decursin (*Angelica gigas* Nakai), decursinol angelate (*Angelica gigas* Nakai), nodakenin (*Angelica gigas* Nakai), formononetin (*Glycyrrhiza uralensis* Fischer), 6-gingerol (*Zingiber officinale* Roscoe), and liquiritin (*Glycyrrhiza uralensis* Fischer) were studied (Fig. 1). Amounts of the 6 marker compounds in Guibi-tang (GB) and fermented Guibi-tang (FGB) were measured by an established HPLC-DAD method. In addition, the effect of GB and FGB on ACE activity was evaluated.

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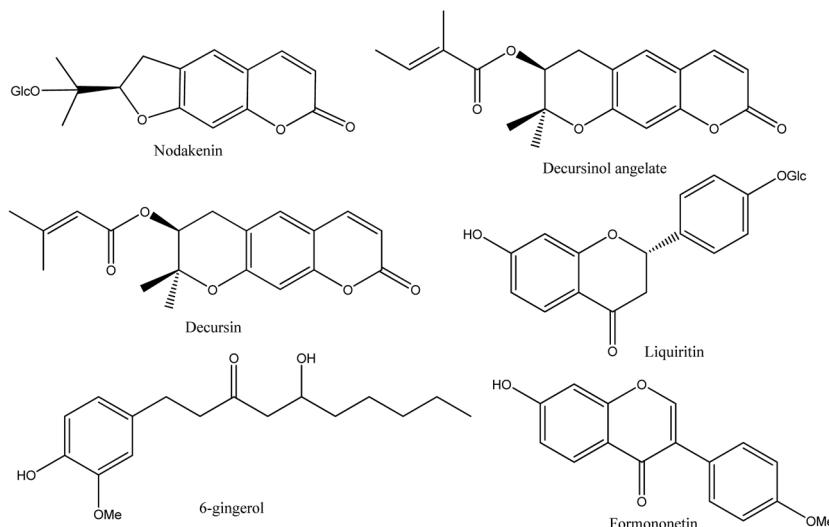


Fig. 1. Chemical structures of 6 constituents in GB.

Experimental

Materials and reagents – Samples of GB powder (3.0 g) were obtained from the Korea Institute of Oriental Medicine. HPLC grade solvents (water and acetonitrile) were purchased from J.T. Baker (USA). The compounds decursin, decursinol angelate, nodakenin and 6-gingerol were purchased from the Korea Food & Drug Administration. Liquiritin was purchased from Wako (Japan), and formononetin was purchased from Sigma-Aldrich (USA). The purities of the 6 standard compounds were greater than 98%. ACE (1 U/ml, rabbit lung) and N-Hippuryl-His-Leu (8.33 mM) were purchased from Sigma-Aldrich (USA).

Fermentation of Guibi-tang – The bacterial strain, *Lactobacillus curvatus* KFRI 166 was obtained from the Korea Food Research Institute (KFRI, Korea). The test organism was transferred into MRS broth for *Lactobacillus* spp. and grown at 37 °C 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 °C. After cooling, 750 ml of GB was combined with 7.5 ml of the *Lactobacillus* inoculum described above. This was incubated at 37 °C for 48 h. A powder of the fermented GB culture was prepared by freeze-drying.

Preparation of samples – Powders of GB (50 mg) and FGB (50 mg) were weighed accurately and dissolved in 1

ml of water. The samples were stored at 4 °C and filtered through a 0.45 µm membrane filter before analysis by HPLC or by bioassay.

Analysis of compounds in GB and FGB – Our 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 mm I.D. × 250 mm) at 40 °C. 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 6 compounds: decursin, decursinol angelate, nodakenin, 6-gingerol, liquiritin and formononetin. The sample injection volume was 20 ml.

Assay for inhibition of ACE activity – ACE activity was assayed by the method of Cushman and Cheung (Cushman and Cheung, 1971) with minor modifications. Briefly, solutions of ACE (8 mU), test sample (0 - 5.0 mg extract/ml) and the ACE substrate, N-Hippuryl-His-Leu were prepared in a borate buffer (100 mM, pH 8.3) containing 0.3 M NaCl. A 50 µl aliquot of ACE solution was pre-incubated with various quantities of GB or FGB in a final volume of 100 µl at 37 °C for 10 min. The mixture was then added to 150 µl of N-Hippuryl-His-Leu

Table 1. Comparison of 6 compounds from GB and FGB

Sample	Content (μg/mg)					
	Decursin	Decursinol angelate	Nodakenin	Formononetin	6-gingerol	Liquiritin
GB	0.099 ± 0.004	0.286 ± 0.01	1.907 ± 0.032	0.072 ± 0.014	0.096 ± 0.021	0.967 ± 0.015
FGB	0.02 ± 0.002	0.012 ± 0.004	1.893 ± 0.226	0.047 ± 0.003	0.087 ± 0.010	0.951 ± 0.052

solution and incubated for a further 60 min at 37 °C. The reaction was stopped by adding 250 μl of 1 N HCl. The sample was mixed with 1.5 mL ethyl acetate to extract hippuric acid, and then centrifuged to separate the ethyl acetate layer. One milliliter of the ethyl acetate layer was then evaporated. The residue was redissolved in distilled water and the amount of extracted hippuric acid was measured by absorbance at 228 nm. The IC₅₀ for each agent's ability to inhibit ACE activity was calculated as the concentration of test sample that inhibited 50% of ACE activity under the experimental conditions. Inhibition of ACE activity was calculated according to the following equation:

$$\text{Inhibition (\%)} = [1 - (S_a - S_b) / C] \times 100$$

where Sa = absorbance of the sample, Sb = absorbance of the blank, and C = absorbance of the control reaction.

Results and discussion

Analysis of compounds in GB and FGB – To determine the effect of fermentation on the concentration of compounds in GB, the 6 compounds of interest - decursin, decursinol angelate, nodakenin, liquiritin, formononetin, and 6-gingerol - were analyzed by HPLC DAD. All compounds were decreased in FGB compared to their levels in GB, with decursin, decursinol angelate, nodakenin, liquiritin, formononetin, and 6-gingerol being decreased by 79.8%, 95.8%, 0.7%, 1.7%, 34.7% and 9.4%, respectively (Table 1). As shown in Table 2, the peak areas of 2 unknown compounds present in GB, (1) and (2), were increased by fermentation. (Fig. 2.)

ACE inhibitory activity of GB and FGB – Fermentation of GB resulted in increased inhibition of ACE activity compared with GB, with IC₅₀ values of 1.729 and 1.011 mg/ml for GB and FGB, respectively (Table 3). Herbal medicines have been used to treat various diseases in Asian countries, and one of these medicines, Guibi-tang is well known for its beneficial effect on cardiovascular disease. Biotransformed GB agents were produced by fermentation with *Lactobacillus curvatus* KFRI 166 to search for metabolites that showed increased biological activity. We hypothesized that some

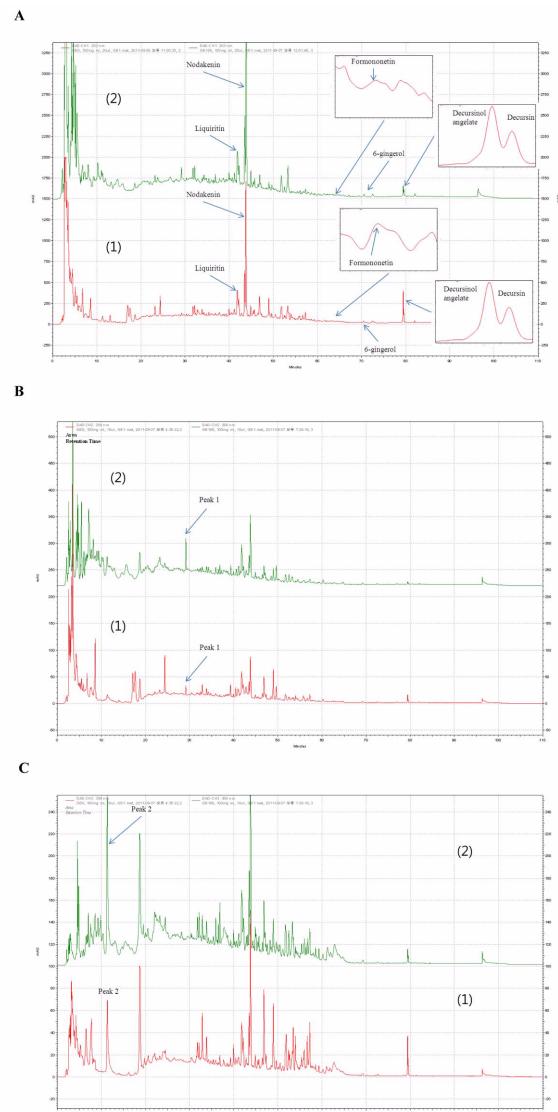


Fig. 2. HPLC chromatograms of GB (1) and FGB (2). (A = 203 nm, B = 254 nm, C = 300 nm).

metabolites in biotransformed GB might show anti-hypertensive effects through modification of their chemical structures. The increased anti-hypertensive effect of biotransformed GB was observed by demonstrating angiotensin-converting enzyme inhibitory activity.

In this study, the amounts of decursin, decursinol angelate, nodakenin, liquiritin, formononetin and 6-

Table 2. Comparison of peak areas of unknown compounds (1) and (2) in GB and FGB

Sample	Peak area	
	Peak 1	Peak 2
GB	2257929	7881918
FGB	4552943	9639919

Table 3. Effects of GB and FGB on angiotensin-converting enzyme activity

Sample	IC ₅₀ (mg/ml)
GB	1.729
FGB	1.011

gingerol in GB and FGB were analyzed. The concentrations of these 6 known compounds and 2 unknown compounds (1 and 2) which were altered during fermentation were determined. Meanwhile, the angiotensin-converting enzyme inhibitory effect became stronger.

In conclusion, we demonstrated that the angiotensin-converting enzyme inhibitory activity of Guibi-tang can be enhanced through fermentation. Further research on converted compounds and newly identified compounds by fermentation and bioactivity of fermented herbal medicines is now required.

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