

Lactobacillus plantarum 발효에 의한 갈근탕의 생물 전환 성분 연구

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Bioconversion Constituents of Galgeun-tang Fermented by *Lactobacillus plantarum*

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ABSTRACT : Galgeun-tang (GGT) is a traditional medicinal formula that is widely prescribed to treat cold, asthma, and hives in Korea. Fermented herbal medicines can be made more effective than normal herbal medicines by increasing the absorption and bioavailability of the active compounds. In this study, we fermented Galgeun-tang to produce bioconversion constituents using *Lactobacillus plantarum* (GGT144), and found that four peaks were decreased, three peaks were increased and two new peaks appeared in the HPLC-DAD chromatogram. After HPLC-DAD-guided fractionation of the newly-appearing compounds (1 and 5) and the increased (6, 7, and 9) compounds, the structure of the compounds was determined using NMR and MS. Using this approach the compounds were identified to be pyrogallol (1), daidzein (5), liquiritigenin (6), cinnamyl alcohol (7), and formononetin (9), respectively. In addition, the decreased compounds were identified to be daidzin (2), liquiritin (3), ononin (4), and cinnam aldehyde (8) using HPLC-DAD analysis with standard compounds. The high performance liquid chromatography method was used to quantify the nine constituents in GGT and GGT144. All calibration curves of the standard compounds displayed excellent linearity with a $R^2 > 0.9968$.

Key Words : Galgeun-tang, Fermentation, *Lactobacillus plantarum*, HPLC-DAD, Calibration Curves

INTRODUCTION

Galgeun-tang (GGT) is a Korea traditional herbal decoction used for the treatment of fever, cold, asthma, inflammation, and hives, and consists of *Pueraria lobata*, *Ephedra sinica*, *Cinnamomum cassia*, *Paeonia lactiflora*, *Glycyrrhiza glabra*, *Zingiber officinale*, and *Zizyphus jujube* (Heo, 1984). Recently, GGT was reported to display some pharmacological effects against osteoporosis (Shim *et al.*, 2011) and act as an anti-inflammatory (Lyu *et al.*, 2006), and antioxidant agent (Shin *et al.*, 2008).

Fermentation of medicinal plants and traditional herbal prescriptions were recently reported to display enhanced biological activity relative to their unfermented counterpart. Total polyphenol and flavonoid contents of *Berberis koreana* were increased by fermentation process (Ji *et al.*, 2010). Chui *et al.* reported that fermented plant extracts hold promise for inducing

apoptosis in human cancer cells (Chui *et al.*, 2006). Jung *et al.* reported that Maesil (*Prunus mume*) fermented with probiotics enhances immune activity in mice (Jung *et al.*, 2010). Jung *et al.* reported that the antioxidant, antidiabetic, and antiobesity activities of fermented aged black garlic (FBG) were superior relative to unfermented black garlic (Jung *et al.*, 2011). You *et al.* reported that Ssanghwa tang fermented with *Saccharomyces cerevisiae* improved exercise performance and reduced fatigue in mice (You *et al.*, 2009). During fermentation of herbs, some glycoside compounds are de-glycosylated into small and hydrophobic aglycones that might be more biologically active than normal herbal medicines due to the increased absorption of the active compounds in the body. Onozawa *et al.* reported that daidzein and genistein inhibited LNCap growth more than glucoside, daidzin, and genistin (Onozawa *et al.*, 1998). Soymilk fermented using probiotics was reported to have a higher isoflavone aglycone content (Wei *et al.*, 2007). Okabe *et al.*

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reported that the isoflavones in aglycone-rich fermented soy were absorbed faster and in greater amounts than those of glucoside-rich soy in postmenopausal Japanese women (Okabe *et al.*, 2011). During fermentation of Green teas, GABA is highly increased by irreversible α -decarboxylation of L-glutamic acid, catalyzed by glutamic acid decarboxylase (GAD) (Hui *et al.*, 2011).

Research on fermented medicinal plants or prescriptions has recently increased, however, very few studies have examined the changes in the constituents after fermentation. In this study, we attempted to ferment Galgeun-tang (GGT). Nine *Lactobacillus* sp. and one *Bifidobacterium* sp., were used for the fermentation of Galgeun-tang. Among the fermented GGT, the one fermented by *L. plantarum* (GGT144) showed the most remarkable compositional changes as analyzed by HPLC-DAD when compared to unfermented Galgeun-tang. Therefore, we analyzed the compositional changes of Galgeun-tang fermented with *L. plantarum* (GGT144) relative to conventional Galgeun-tang using a HPLC-DAD system and various chromatographic isolation techniques. In this paper, we report the bioconversion compositions of fermented Galgeun-tang (GGT144), which was analyzed using HPLC-DAD, MS, and NMR.

MATERIALS AND METHODS

1. General experimental procedures

The NMR spectra were obtained using an INOVA 600 and INOVA 400NB (Varian Co., USA), and low-resolution JMS-600W FAB-MS (JEOL Co., Japan). The analytical and semi-preparative HPLC data were obtained using a L-2130 pump, L-2200 auto-sampler, L-2350 column oven, L-5200 fraction collector, and L-2455 diode array UV/VIS detector (Elite Lachrom HPLC system, Hitachi Co., Japan). The semi-preparative HPLC columns used were OptimaPak C₁₈ (4.6 mm × 250 mm, 5 μ m, RS tech Co., Korea), Agilent prep-C₁₈ (21.2 × 250 mm, 10 μ m, Agilent Co., USA), and Agilent prep-Si (21.2 × 250 mm, 10 μ m, Agilent Co., USA). For analysis of bioconversion constituents, an OptimaPak C18 column (5 μ m, 100 Å, 4.6 mm × 250 mm, RS tech Co., Korea) was used and the DAD UV wavelength was 198, 250, 275, and 290 nm. The mobile phase consisted of water and acetonitrile with a gradient elution at a flow rate of 1.0 mL/min (Table 1). The column temperature was maintained at 40°C. The injection volume of the samples was 10 μ L. Open column chromatography was performed using C₁₈ powder (YMC*Gel ODS-A, particle size 75 μ m, YMC Co., Ltd., Japan).

Table 1. Mobile phase condition of chromatographic separation.

Time (min)	Water	Acetonitrile	Flow rate (mL/min)
0	95	5	1
5	95	5	1
70	0	100	1
80	0	100	1

2. Materials and reagents

The root of *Pueraria lobata* (Korea), stem of *Ephedra sinica* (Korea), bark of *Cinnamomum cassia* (Vietnam), root of *Paenonia lactiflora* (Korea), root of *Glycyrrhiza glabra* (Vietnam), root of *Zingiber officinale* (Korea), and fruit of *Zizyphus jujube* (Korea) were purchased from the Korea Medicine Herbs Association (Yeongcheon, Korea). Daidzin and liquiritin were purchased from Wako Pure Chemical Industries, Ltd (Japan). Cinnamaldehyde and ononin were purchased from Sigma Chemical Co. (USA). HPLC grade solutions, water, metanol, and acetonitrile, were purchased from J.T. Baker Co. (USA).

3. Materials and reagents

The root of *Pueraria lobata* (Korea), stem of *Ephedra sinica* (Korea), bark of *Cinnamomum cassia* (Vietnam), root of *Paenonia lactiflora* (Korea), root of *Glycyrrhiza glabra* (Vietnam), root of *Zingiber officinale* (Korea), and fruit of *Zizyphus jujube* (Korea) were purchased from the Korea Medicine Herbs Association (Yeongcheon, Korea). Daidzin and liquiritin were purchased from Wako Pure Chemical Industries, Ltd (Japan). Cinnamaldehyde and ononin were purchased from Sigma Chemical Co. (USA). HPLC grade solutions, water, metanol, and acetonitrile, were purchased from J.T. Baker Co. (USA).

4. Preparation of Galgeun-tang (GGT)

The root of *P. lobata* (400 g, 32.0%), stem of *E. sinica* (200 g, 16.0%), bark of *C. cassia* (150 g, 12.0%), root of *P. lactiflora* (150 g, 12.0%), root of *G. glabra* (100 g, 8.0%), root of *Z. officinale* (50 g, 4.0%), and fruit of *Z. jujube* (200 g, 16.0%) were prepared in an extractor (Cosmos-600 Extractor, Gyeongseo Co., Inchon, Korea) and diluted 10-fold with water. After refluxing for three hours and cooling, the samples were filtered to produce GGT.

5. Fermentation of Galgeun-tang (GGT144)

The GGT was adjusted to pH 7.0 using 1 M NaOH, and was then sterilized in an autoclave at 120°C for 15 min and cooled to

37°C. The GGT was fermented by the inoculation of 167 mL of *L. plantarum* KFRI144 (1×10^8 CFU/mL). Pure cultures of *L. plantarum* were obtained from the Korea Food Research Institute (KFRI). Before experimental use, the bacterial strain was incubated in 50 mL of MRS medium (Difco™ Lactobacilli MRS Broth, Becton Dickinson and Company, Hunt Valley, MD, USA) at 37°C overnight. The GGT was fermented with *L. plantarum* at 37°C for 48 h and then filtered through a 60- μ m nylon net filter (Millipore, Billerica, MA, USA), lyophilized, and stored in desiccators at 4°C before use.

6. Preparation of standard solutions and samples

Each standard solution was prepared by dissolving standard compounds (daidzin, liquiritin, cinnamaldehyde, and ononin) in 100% methanol at a concentration of 250 μ g/mL. To prepare the analytical samples, the GGT and GGT144 powder was weighed and dissolved in 100% H₂O at a concentration of 20 mg/mL. Prior to analysis, the sample preparation was filtered through a 0.45 m filter.

7. Solvent fractionation of GGT144 and isolation of bioconversion constituents

The GGT144 (16 L) was partitioned with equal volumes of EtOAc and *n*-BuOH, successively. All of the fractions were evaporated to dryness, giving EtOAc (15 g), *n*-BuOH (60 g), and water (340 g) soluble fractions. Ethyl acetate fraction (15.0 g) was subjected to C18 flash column chromatography (100% H₂O, 20%, 40%, 60%, 80, and 100% MeOH) to produce seven fractions (F1-F7). F1 (1.2 g) was purified using C18 open column chromatography with 100% H₂O to produce **1** (30 mg). F5 (5.0 g) was purified using C18 flash column chromatography (60% MeOH) to produce seven fractions (F51-F57). F53 (2.0 g) was isolated using C18 open column chromatography with 55% MeOH and purified by semi-preparative Si HPLC (chloroform/methanol, 20 : 1) to afford **5** (12 mg). F54 (1.0 g) was isolated by C18 open column chromatography with 50% MeOH and purified using semi-preparative Si HPLC (chloroform/methanol, 30 : 1) to produce **6** (4 mg). F56 (1.0 g) was isolated by C18 open column chromatography with 55% MeOH and purified using semi-preparative Si HPLC (*n*-hexane/EtOAc, 7 : 1) to afford **7** (20 mg). F6 (3.0 g) was purified using semi-preparative C18 HPLC (60% MeOH) repeatedly to produce **9** (1 mg).

Pyrogallol (1) : White powder; EI-MS *m/z* 126 [M]⁺; ¹H-NMR (DMSO-*d*₆, 400 MHz) δ 6.30 (2H, d, *J*=8.4 Hz, H-3, 5), 6.48 (1H, dd, *J*=8.4, 7.6 Hz, H-4); ¹³C-NMR (DMSO-*d*₆,

100 MHz) δ 134.5 (C-1), 147.7 (C-2, 6), 108.5 (C-3, 5), 119.8 (C-4).

Daidzein (5) : Yellow powder; EI-MS *m/z* 254 [M]⁺; ¹H-NMR (DMSO-*d*₆, 600 MHz) δ 8.34 (1H, s, H-2), 8.03 (1H, d, *J*=8.4 Hz, H-5), 7.45 (2H, d, *J*=8.6 Hz, H-2', 6'), 7.00 (1H, dd, *J*=8.4, 2.4 Hz, H-6), 6.93 (1H, d, *J*=2.4 Hz, H-8), 6.87 (2H, d, *J*=9.0 Hz, H-3', 5'); ¹³C-NMR (DMSO-*d*₆, 100 MHz) δ 153.5 (C-2), 123.1 (C-3), 175.4 (C-4), 127.9 (C-5), 115.8 (C-6), 163.2 (C-7), 102.7 (C-8), 157.8 (C-9), 117.3 (C-10), 124.1 (C-1'), 130.7 (C-2'), 115.6 (C-3'), 157.8 (C-4'), 115.6 (C-5'), 130.7 (C-6')

Liquiritigenin (6) : Yellow powder; EI-MS *m/z* 256 [M]⁺; ¹H-NMR (CD₃OD, 600 MHz) δ 2.70 (1H, ddd, *J*=16.8, 3.0, 1.8 Hz, H-3a), 3.07 (1H, ddd, *J*=16.8, 13.2, 1.8 Hz, H-3b), 5.39 (1H, dd, *J*=13.2, 2.4 Hz, H-2), 6.37 (1H, d, *J*=1.8 Hz, H-8), 6.52 (1H, dd, *J*=9.0, 1.8 Hz, H-6), 6.84 (2H, dd, *J*=8.4, 1.8 Hz, H-3', 5'), 7.34 (2H, d, *J*=8.4 Hz, H-2', 6'), 7.75 (1H, dd, *J*=8.4, 1.4 Hz, H-5); ¹³C-NMR (CD₃OD, 100 MHz) δ 43.8 (C-3), 79.8 (C-2), 102.6 (C-8), 110.5 (C-6), 113.7 (C-10), 115.1 (C-3', 5'), 127.8 (C-2', 6'), 128.7 (C-5), 130.1 (C-1'), 157.8 (C-4'), 164.4 (C-9), 165.6 (C-7), 192.4 (C-4).

Cinnamyl alcohol (7) : Colorless oil; EI-MS *m/z* 134 [M]⁺; ¹H-NMR (CD₃OD, 600 MHz) δ 7.39 (2H, d, *J*=7.3 Hz, H-2, 6), 7.28 (2H, dd, *J*=7.3, 7.3 Hz, H-3, 5), 7.19 (1H, dd, *J*=7.3, 7.3 Hz, H-4), 6.59 (1H, d, *J*=15.8 Hz, H-7), 6.35 (1H, dt, *J*=15.8, 5.6 Hz, H-8), 4.22 (2H, d, *J*=5.6 Hz, H-9); ¹³C-NMR (CD₃OD, 100 MHz) δ 137.2 (C-1), 130.4 (C-7), 128.7 (C-4), 128.4 (C-3, 5), 127.3 (C-8), 126.2 (C-2, 6), 62.5 (C-9).

Formononetin (9) : Yellow powder; ESI-MS *m/z* 268 [M]⁺; ¹H-NMR (DMSO-*d*₆, 400 MHz) δ 3.74 (3H, s, OCH₃), 6.83 (1H, d, *J*=1.8 Hz, H-8), 6.90 (1H, dd, *J*=9.0, 1.8 Hz, H-6), 6.95 (2H, d, *J*=9.0 Hz, H-3', 5'), 7.47 (2H, d, *J*=9.0 Hz, H-2', 6'), 7.93 (1H, d, *J*=9.0 Hz, H-5), 8.30 (1H, s, H-2); ¹³C-NMR (DMSO-*d*₆, 100 MHz) δ 153.8 (C-2), 124.9 (C-3), 175.3 (C-4), 128.0 (C-5), 115.8 (C-6), 163.2 (C-7), 102.8 (C-8), 158.1 (C-9), 117.2 (C-10), 123.8 (C-1'), 130.7 (C-2'), 114.2 (C-3'), 158.1 (C-4'), 114.2 (C-5'), 130.7 (C-6'), 55.8 (OCH₃)

RESULTS AND DISCUSSION

Five bioconversion constituents (**1**, **5**, **6**, **7**, and **9**) were isolated and purified from Galgeun-tang fermented with *L. plantarum* KFRI144. Two of the compounds (**1** and **5**) were newly appeared, three (**6**, **7**, and **9**) had higher intensities and four (**2**, **3**, **4**, and **8**) had lower intensities after fermentation. Four constituents (**2**, **3**, **4**, and **8**) with decreased intensities were analyzed and

Table 2. Standard curves and R² value of 1-9.

Components	Retention time (t _R , min)	Equation ^a	R ² (n = 5)
Pyrogallol (1)	7.14	y = 733582.25x - 2155747.77	0.9968
Daidzin (2)	20.15	y = 142869x + 99161	0.9998
Liquiritin (3)	22.27	y = 70263x - 30546	0.9997
Ononin (4)	27.02	y = 138795x + 77828	0.9998
Daidzein (5)	28.38	y = 246057x + 32031	1.0000
Liquiritigenin (6)	29.00	y = 42773x + 3879.6	1.0000
Cinnamyl alcohol (7)	30.07	y = 293867x + 198133	1.0000
Cinnamaldehyde (8)	33.67	y = 376117x + 96490	1.0000
Formononetin (9)	36.24	y = 265663x + 1424.3	1.0000

^ay: peak area, x:concentration (μg/mL)

Table 3. Content of bioconversion constituents (1-9) in GGT and GGT144.

Components	Retention time (t _R , min)	GGT (μg/mL)	GGT144 (μg/mL)	Ratio ^a (μg/mL)	Ratio ^a (%)
Pyrogallol (1)	7.14	0.000	11.582	+11.582	Newly appeared
Daidzin (2)	20.15	99.917	57.026	-42.891	-42.9
Liquiritin (3)	22.27	37.105	11.409	-25.696	-69.3
Ononin (4)	27.02	20.316	5.706	-14.610	-71.9
Daidzein (5)	28.38	0.000	27.925	+27.925	Newly appeared
Liquiritigenin (6)	29.00	4.136	45.856	+41.720	1008.7
Cinnamyl alcohol (7)	30.07	6.434	34.830	+28.396	441.3
Cinnamaldehyde (8)	33.67	76.572	27.186	-49.386	-64.5
Formononetin (9)	36.24	0.232	5.445	+5.213	2247

^aContents changes of bioconversion constituents after fermentation of GGT

compared with standard compounds. All of the compounds (1-9) in GGT and GGT144 were then quantitatively analyzed (Table 2 and 3). The HPLC-DAD analysis conditions were successfully established to separate the GGT and GGT144 peaks (Fig. 1-4.). Four decreased peaks were observed at retention times of 20.15, 22.27, 27.02, and 33.67 min, three increased peaks were observed at 29.00, 30.07, and 36.24 min, and two newly appearing peaks were observed at 7.14 and 28.38 min.

To isolate the peaks with increased intensities and the newly appearing peaks, GGT144 in water was partitioned with EtOAc, followed by *n*-BuOH. The EtOAc, *n*-BuOH, and water fractions were analyzed by HPLC-DAD and all of the increased peaks, newly appearing and decreased peaks were found in the ethyl acetate fraction. HPLC-DAD-guided fractionation of the EtOAc fraction produced five pure compounds, and their structures were identified by MS, ¹H-, and ¹³C-NMR and compared with published data (Kinjo *et al.*, 1987; Pouchert and Behnke J, 1993a; Pouchert and Behnke, 1993b; Kanakubo *et al.*, 2001; Choi *et al.*, 2010).

The newly-appearing constituents at 7.14 and 28.38 min were

identified as pyrogallol (1) and daidzein (5). The increased constituents at 29.00, 30.07, and 36.24 min were determined to be liquiritigenin (6), cinnamyl alcohol (7), and formononetin (9). The decreased constituents at 20.15, 22.27, 27.02, and 33.67 min were identified as daidzin (2), liquiritin (3), ononin (4), and cinnamaldehyde (8) by comparison with the retention time (t_R) and DAD spectra of standard compounds.

Pyrogallol (1, t_R 7.14 min, Fig. 4.) and daidzein (5, t_R 28.38 min, Fig. 2.) newly appeared in GGT144 (11.582 and 27.925 μg/mL). Since glycoside of 5, daidzin (2, t_R 20.15 min, 99.917 μg/mL in GGT, Fig. 2.), decreased by approximately 42.9% (57.026 μg/mL), compound 5 was assumed to be produced from daidzin (2). Glycosides of pyrogallol (1) were not detected in GGT.

Liquiritigenin (6, t_R 29.00 min, Fig. 1.), cinnamyl alcohol (7, t_R 30.07 min, Fig. 2.), and formononetin (9, t_R 36.24 min, Fig. 2.) were remarkably increased in GGT144 (45.856, 34.830, and 5.445 μg/mL) when compared to GGT (4.136, 6.434, and 0.232 μg/mL), while liquiritin (3, t_R 22.27 min, Fig. 1.), cinnamaldehyde (8, t_R 33.67 min, Fig. 3.), and ononin (4, t_R 27.02

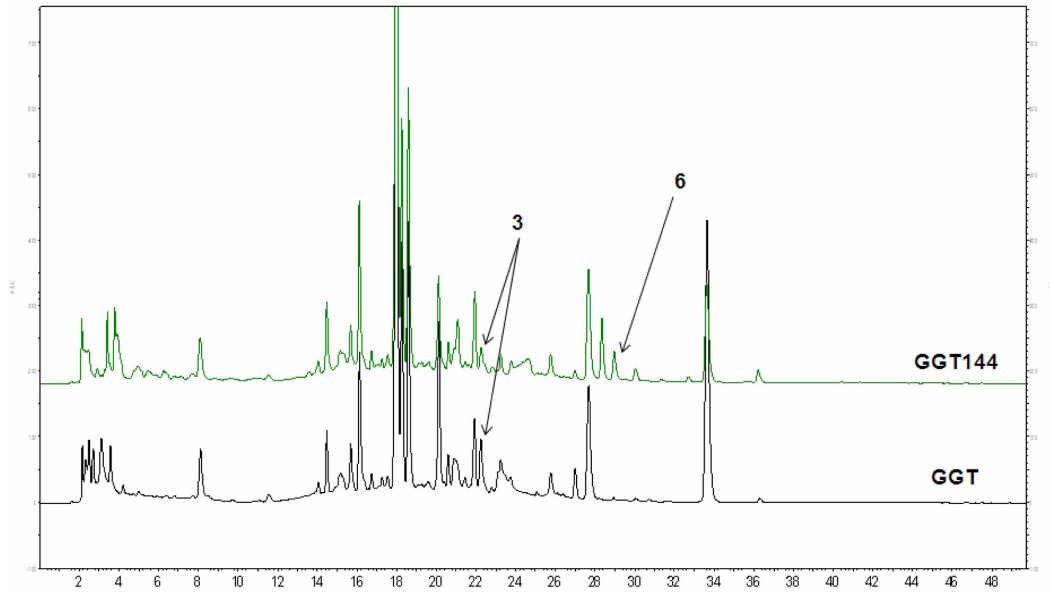


Fig. 1. HPLC chromatograms of Galgeun-tang (GGT) and fermented Galgeun-tang (GGT144) at 275 nm. 3. Liquiritin, t_R 22.27 min; 6. Liquiritigenin, t_R 29.00 min.

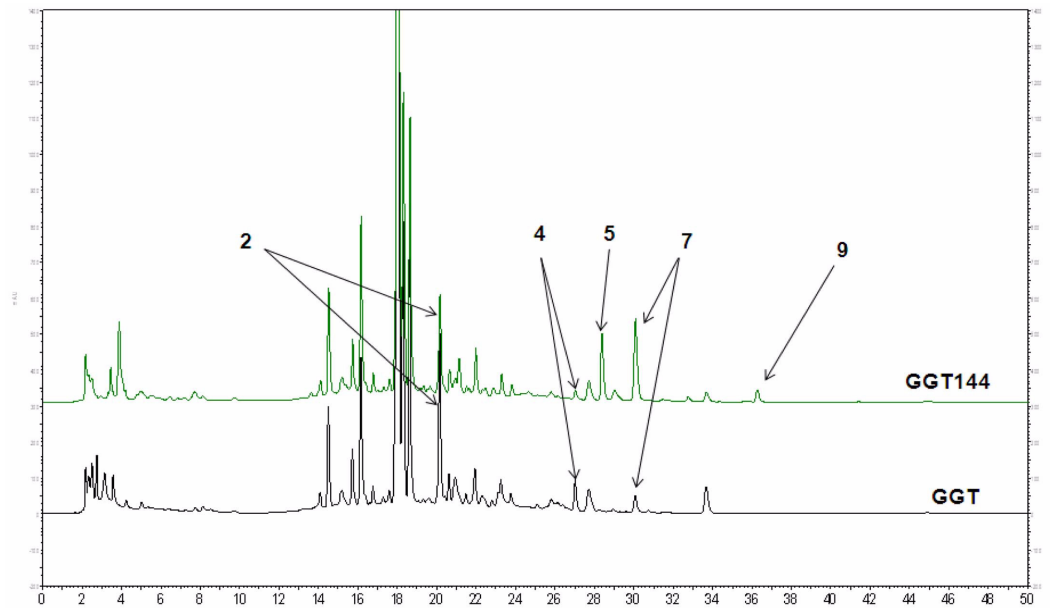


Fig. 2. HPLC chromatograms of Galgeun-tang (GGT) and fermented Galgeun-tang (GGT144) at 250 nm. 2. Daidzin, t_R 20.15 min; 4. Ononin, t_R 27.02 min; 5. Daidzein, t_R 28.38 min; 7. Cinnamyl alcohol, t_R 30.07 min; 9. Formononetin, t_R 36.24 min.

min, Fig. 2.) were decreased in GGT144 (11.409, 27.186, and 5.706 $\mu\text{g/mL}$) when compared to GGT (37.105, 76.572, and 20.316 $\mu\text{g/mL}$). Therefore, 3, 8, and 4 were assumed to be transformed into liquiritigenin (6, Fig. 1.), cinnamyl alcohol (7, Fig. 2.), and formononetin (9, Fig. 2.).

The original plant of pyrogallol (1) is still not known, and

pyrogallol was previously reported to have antitumor (Yang *et al.*, 2009) and antioxidant activity (Park *et al.*, 2007). Pyrogallol (1) was assumed to be produced by the deglycosylation of tannic acid. Nelson *et al.* and Odenyo *et al.* reported that pyrogallol was the predominant fermentation product of tannic acid and also suggested that pyrogallol was produced by intergallate depside

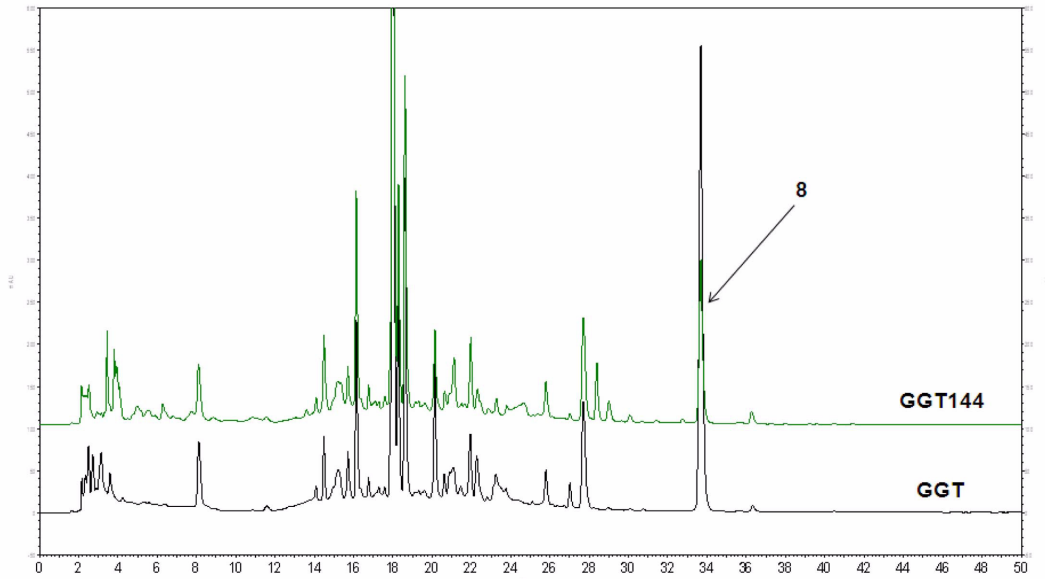


Fig. 3. HPLC chromatograms of Galgeun-tang (GGT) and fermented Galgeun-tang (GGT144) at 290 nm. 8. Cinnamaldehyde, t_R 33.67 min.

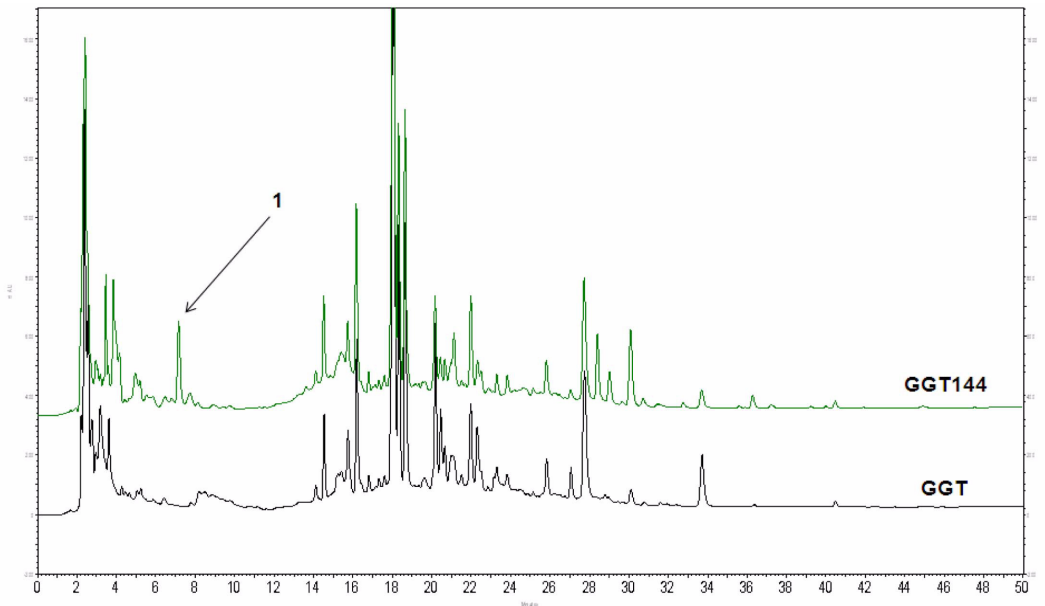


Fig. 4. HPLC chromatograms of Galgeun-tang (GGT) and fermented Galgeun-tang (GGT144) at 198 nm. 1. Pyrogallol, t_R 7.14 min.

linkages and the ester bond between gallate and glucose are both hydrolyzed, resulting in gallate decarboxylation (Nelson *et al.*, 1995; Odenyo and Osuji, 1998). Rodríguez *et al.* reported that *L. plantarum* produced pyrogallol through the degradation of tannic acid. In nature, tannins are found in many different families of higher plants (Rodríguez *et al.*, 2008). Based on these previous studies, we deduced that the original plant of pyrogallol was all

of the herbs of Galgeun-tang.

Daidzein (5) was assumed to be produced by the deglycosylation of daidzin from the root of *P. lobata* and was reported to have anti-diabetic effects (Park and Ko, 2006), antitumor properties (Choi and Kim, 2008) and to stimulate osteoblastic bone formation (Sugimoto and Yamaguchi, 2000).

Liquiritigenin (6) was assumed to be produced by the

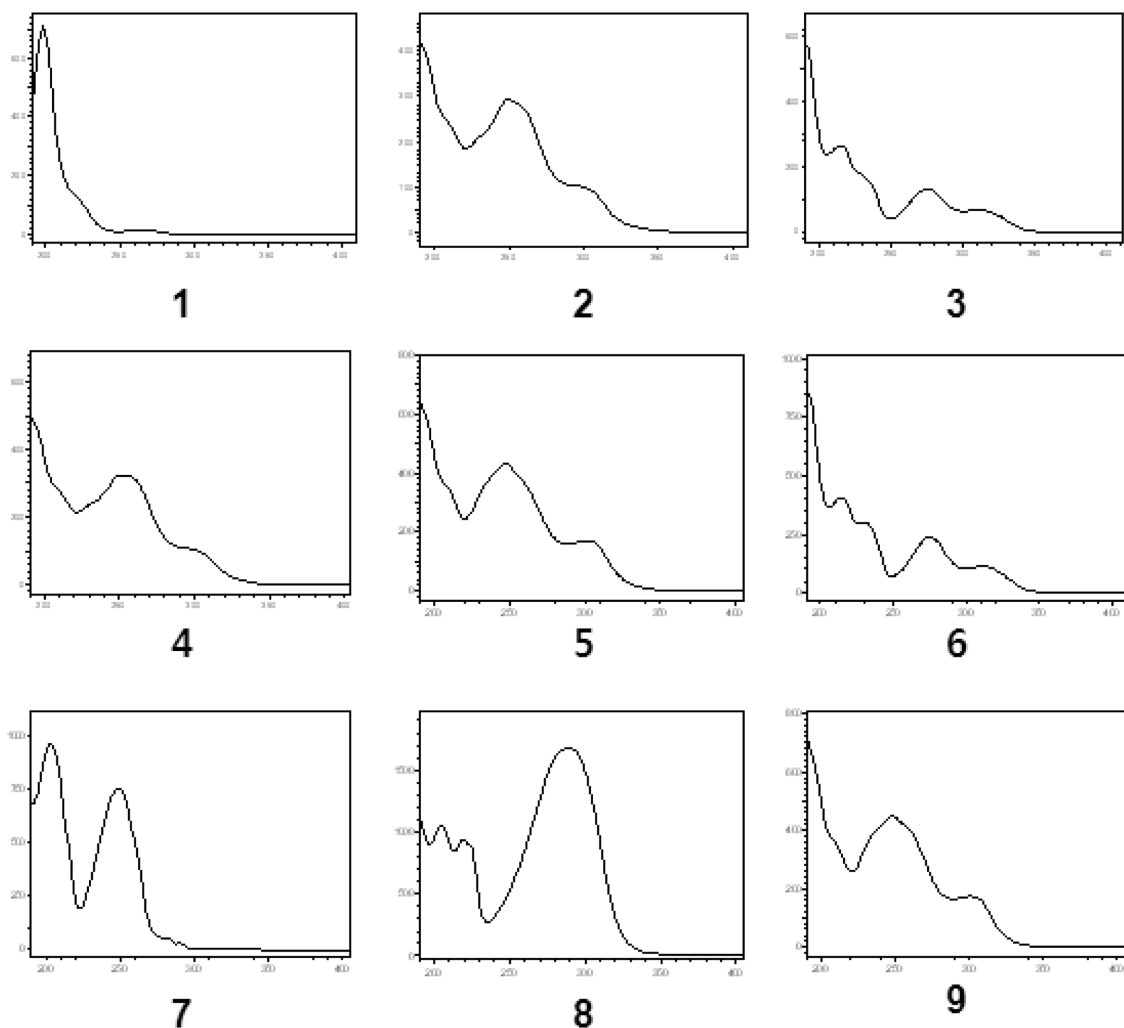


Fig. 5. HPLC DAD spectrums of compounds 1-9. 1. Pyrogallol; 2. Daidzin; 3. Liquiritin; 4. Ononin; 5. Daidzein; 6. Liquiritigenin; 7. Cinnamyl alcohol; 8. Cinnamaldehyde; 9. Formononetin.

deglycosylation of liquiritin from the root of *G. glabra*, and was reported to have antibacterial activity (Park *et al.*, 2009), antitumor effects (Zhang *et al.*, 2009), and neuroprotective effects (Liu *et al.*, 2010).

Cinnamyl alcohol (7) was assumed to be produced by the hydrogenation of cinnamaldehyde (8) from *Cinnamomum cassia*, and was reported to have UV-blocking effect (Addo *et al.*, 1982).

Cinnamyl alcohol (7) increased by about 441.3% after fermentation, while cinnamaldehyde (8) decreased by approximately 64.5% after fermentation of GGT. This result also indicates that cinnamyl alcohol (7) was produced by the hydrogenation of cinnamaldehyde. Zucca *et al.* reported the biotransformation of cinnamaldehyde to cinnamyl alcohol by alcohol dehydrogenase (ADH) (Zucca *et al.*, 2009) and

production of cinnamyl alcohol (6) was assumed to occur through hydrogenation by ADH from the *L. plantarum*.

Formononetin (9) was assumed to be produced by the deglycosylation of ononin from the root of *P. lobata*, and was reported to have vasorelaxation (Wu *et al.*, 2010), antioxidant, and estrogenic effects (Mu *et al.*, 2009).

Izumi *et al.* and Okabe *et al.* reported that soy isoflavone aglycones were absorbed faster and in higher amounts than their glucosides in humans and the bioavailability of the aglycone form of isoflavone was found to be higher than the glycoside form (Izumi *et al.*, 2000; Okabe *et al.*, 2011). Remarkable bioavailability and bioactivity was also reported in foods and medicinal plants containing bioactive aglycone, such as fermented soybeans (Okabe *et al.*, 2011), fermented soymilk

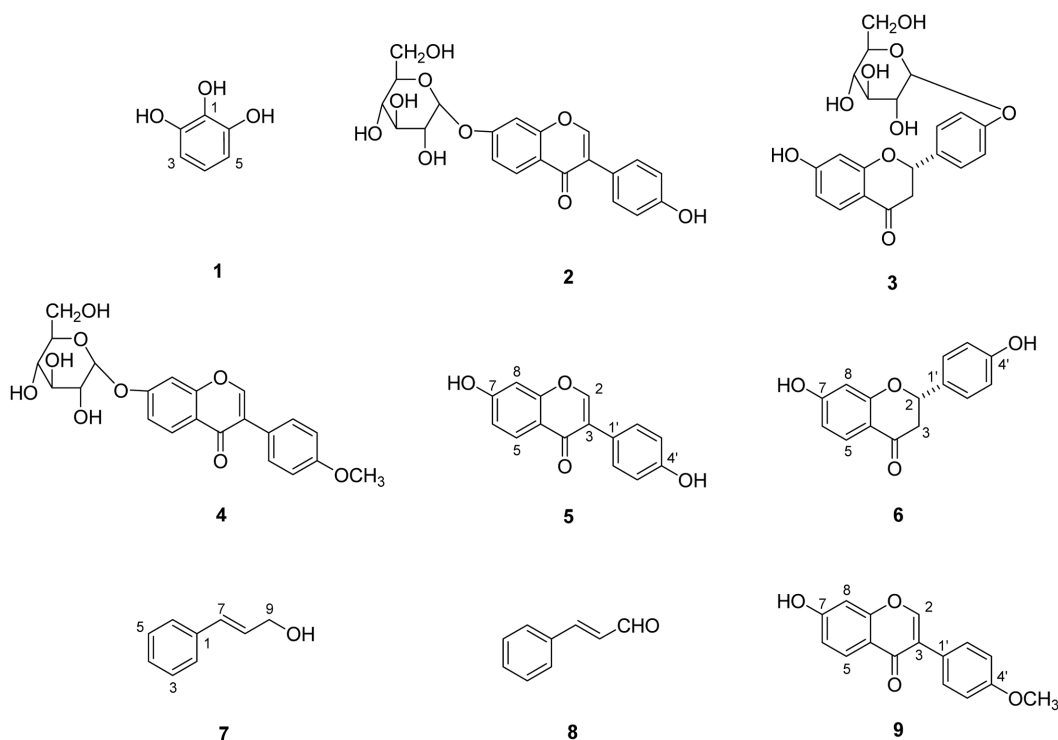


Fig. 6. Structures of compounds from GGT144. 1. Pyrogallol; 2. Daidzin; 3. Liquiritin; 4. Ononin; 5. Daidzein; 6. Liquiritigenin; 7. Cinnamyl alcohol; 8. Cinnamaldehyde; 9. Formononetin.

(Kano *et al.*, 2006), and fermented red ginseng (Kim *et al.*, 2010). Kuwaki *et al.* found that the antifungal activity of fermented herb formulas against tinea was as high as that of a synthetic fungicide (Kuwaki *et al.*, 2002). Kwak *et al.* reported that the antioxidant activity of Chungkookjang (a Korea fermented soybean paste) was stronger than unfermented steamed soybeans (Kwak *et al.*, 2007). Kitawaki *et al.* reported that soy yogurt decreased the hepatic triglyceride content in rats (Kitawaki *et al.*, 2009).

In conclusion, because of the production of pyrogallol (1), daidzein (5), liquiritigenin (6), cinnamyl alcohol (7), and formononetin (8) during the fermentation of GGT, GGT144 could be increased antitumor, antibacterial, vasorelaxation, antioxidant, estrogenic, or neuroprotective effect. In addition, it is expected that this study will help to establish quality control parameters for GGT144.

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