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열수추출 과정에서 삽주, 백출(큰꽃삽주), 북창출 배합이 감초 성분의 추출률에 미치는 영향

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Chemical influences of the rhizomes of *Atractylodes japonica*, *A. macrocephala*, or A. chinensis on the extraction efficiencies of chemical compounds in the roots and rhizomes of *Glycyrrhiza uralensis* during hot-water extraction

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ABSTRACT

Objectives : When herbal medicines are extracted together, they may interact with each other, leading to change of chemical characteristics. This study aimed to evaluate the influence of *Atractylodes* rhizomes (*Atractylodes japonica*, *A. macrocephala*, and *A. chinensis*) on the chemical features of the roots and rhizomes of *Glycyrrhiza uralensis*, which is are commonly combined with herbal medicines in many herbal formulae, when they are co-decocted.

Methods : Liquiritin apioside, liquiritin, ononin, and glycyrrhizin levels of *G. uralensis* in hot-water extracts prepared by the combination of *Atractylodes* rhizomes with various weight ratios (*G. uralensis* : *Atractylodes* rhizomes = 10:0, 10:5, 10:10, and 10:20) and extraction times (60, 90, and 120 min) were quantified using a HPLC-diode array detector and compared by statistical analysis.

Results : The concentrations of liquiritin apioside, liquiritin, ononin, and glycyrrhizin from *G. uralensis* roots and rhizomes mostly reduced when co-extracted with *Atractylodes* rhizomes, and the addition of *A. chinensis* most reduced their contents between *Atractylodes* combination groups. *A. japonica* and *A. macrocephala* rhizomes also showed differences of liquiritin and glycyrrhizin levels at 10 g and 20 g groups of Atractylodes rhizomes. Extraction times also affected the concentrations of liquiritin, ononin, and glycyrrhizin mostly during 60 and 90 min.

Conclusions : *Atractylodes* rhizomes might alter the chemical characteristics of *G. uralensis* when these herbs are co-decocted. This study provides the understanding of the chemical interactions of herbal medicines during the extraction in hot water.

Key words : *Atractylodes japonica*, *A. macrocephala*, *A. chinensis*, *Glycyrrhiza uralensis*, chemical interaction, hot-water extraction

I. Introduction

The compositional principle of herbal formulae usually begins with the combination of herbal medicines containing various active constituents and interactions between these herbs may determine their therapeutic effects. The extraction efficiencies of herbal medicines are largely determined by the amounts of compounds

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extracted from herbs under specific conditions, and herbal combinations generally change compound extraction efficiencies[1-3].

The rhizomes of *Atractylodes japonica* Koidz. ex Kitam., *A. macrocephala* Koidz., and *A. chinensis* (Bunge) Koidz. are used as traditional gastrointestinal medicines and belong to different medicinal application categories: the rhizomes of *A. chinensis* are used as 'Changchul'; those of *A. macrocephala* as 'Baekchul'; and those of *A. japonica* as 'Changchul' or 'Baekchul'[4-6]. The roots and rhizomes of *Glycyrrhiza uralensis* Fisch. are commonly used as an essential element to prepare herbal formulas or are used alone to treat diverse diseases. *Atractylodes* rhizomes can be complexed with various herbal medicines for different medicinal applications, but different species of the *Atractylodes* genus are commonly used in combination with *G. uralensis*[7-9].

Accordingly, interactions between different species of the genus *Atractylodes* and *G. uralensis* might crucially influence the therapeutic effect of herbal formulae because the chemical constituents of *Atractylodes* rhizomes exhibit species-specific differences[10,11]. Therefore, it might be an interesting question whether combinations of different *Atractylodes* species could differentially affect chemical characteristics of *G. uralensis* prepared by co-decoction. However, few studies have addressed the effect of combination treatments based on these types of herbal medicines[12].

Therefore, in the present study, the combined effects of three species of *Atractylodes*, that is, *A. japonica*, *A. macrocephala*, or *A. chinensis* on the extraction efficiencies of four marker compounds (liquiritin apioside, liquiritin, ononin, and glycyrrhizin), which were generally selected for the markers[13,14], in *G. uralensis* roots and rhizomes were compared in hot-water extraction.

II. Materials and Methods

1. Chemicals and reagents

Acetonitrile, methanol and water were purchased from J.T. Baker Inc. (HPLC grade; Phillipsburg, NJ, USA). Trifluoroacetic acid and liquiritin were purchased from Sigma-Aldrich (St Louis, MO, USA). Liquiritin apioside was purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, Sichuan, China). Ononin and glycyrrhizin were purchased from ChemFace (Wuhan, Hubei, China). All marker compounds had purities of ≥ 98% The chemical structures of all marker compound and IS are shown in Figure 1.

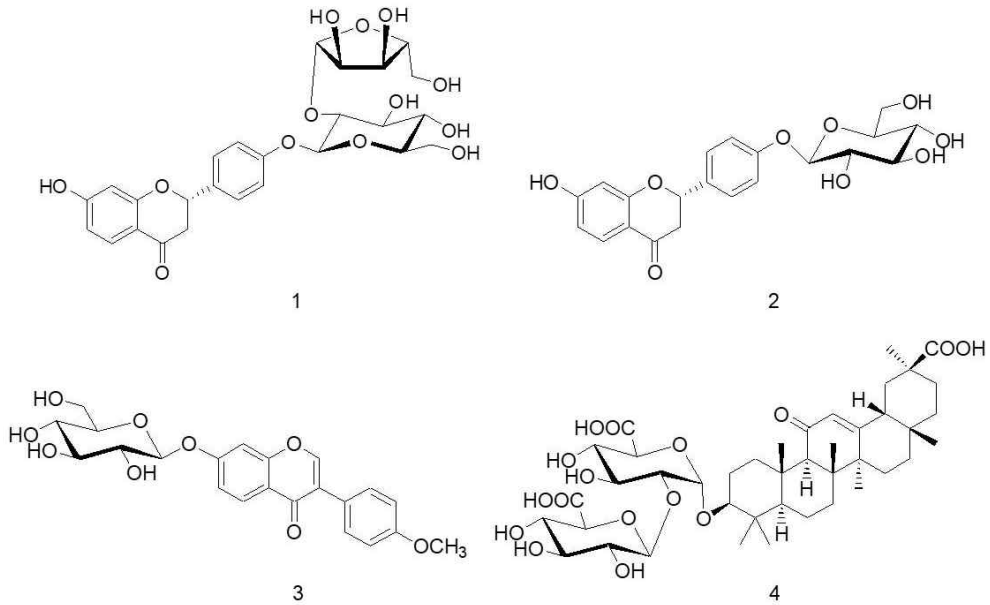

Fig. 1. Chemical structures of the marker compounds. 1, Liquiritin apioside; 2, liquiritin; 3, ononin; 4, glycyrrhizin.

Dried rhizomes of *Atractylodes japonica* Koidz. ex Kitam., *A. macrocephala* Koidz., and *A. chinensis* (Bunge) Koidz. and dried roots and rhizomes of *G. uralensis* Fisch. were purchased from Kwangmyungdang Medicinal Herbs (Ulsan, Korea) and these herbs were authenticated by the author (J.-H. Kim). Voucher specimens (2018-PNUKM-AJ01, -AM01, -AC01, and -GU01) have been deposited at the School of Korean Medicine, Pusan National University.

2. Sample preparation

Combinations of *G. uralensis* (10 g) and *A. japonica*, *A. macrocephala*, or *A. chinensis* rhizomes (the 5, 10, and 20 g groups, respectively) were extracted with 500 ㎖ of distilled water (*w/v*) at 100 ℃ for 120 min using a heat-reflux extractor.

In addition, *G. uralensis* (10 g) and each of the three *Atractylodes* species (10 g) were extracted as mentioned above, but with different extraction times (the 60, 90, and 120 min groups, respectively).

Hot-water extracts were transferred to volumetric flasks and made up to 500 ㎖ with distilled water, and filtered through 0.2 ㎛ syringe filters (BioFact, Daejeon, Korea). Filtrates (200 ㎕) were then prepared for HPLC analysis by adding HPLC grade water (800 ㎕) to make a final solution.

3. HPLC analytical conditions

An Agilent 1260 liquid chromatography system (Agilent Technologies, Palo Alto, CA, USA) equipped with an autosampler, degasser, quaternary solvent pump, and diode array detector was used for the quantitative analysis. Data were processed using Chemstation software (Agilent Technologies Inc., USA). Four marker compounds, liquiritin apioside, liquiritin, ononin, and glycyrrhizin, were separated on a Capcell Pak Mg II C_{18} column(4.6 ㎜ × 250 ㎜, 5 ㎛ ; Shiseido, Tokyo, Japan) at 35℃ using flow rate of 1 ㎖/min and an injection volume of 10 ㎕. The mobile phase consisted of water containing 0.1% TFA (solvent A) and acetonitrile (solvent B). Elution was conducted using a gradient elution program as follows: 15% (B) for 0-3 min, 15-40% (B) for 3-35 min, 40% (B) for 1 min, 40-80% (B) for 36-42 min, 80% (B) for 1 min, and then re-equilibrated to 15% (B) until the end of the analysis. The diode-array detector was set at ultraviolet wavelengths of 250 and 275 ㎚.

4. Validation of the HPLC method

The four marker compounds were accurately weighed and dissolved in methanol at 1000 ㎍/㎖ to make stock solutions, and then serially diluted to produce working solutions of different concentrations. Working solutions were injected into the HPLC to construct calibration curves. The linearities of calibration curves were evaluated using correlation coefficients (r^2). LOD and LOQ were defined as signal-to-noise (S/N) ratio of 3 and 10, respectively.

The precisions were determined by analyzing low, medium, and high concentrations of the above-mentioned working solutions three times within a day (intraday precision) and over three consecutive days (interday precision). Precisions are represented as relative standard deviations (RSDs), where RSD (%) = [(standard deviation / mean)×100].

The accuracy of the HPLC method was determined by testing the recoveries of three known amounts of the six marker compounds (low, medium, and high concentrations) added to sample solutions. Recovery was calculated as follows: Recovery (%) = [(detected concentration − initial concentration) / spiked concentration] × 100.

5. Statistical analysis

Multiple comparisons of different contents of liquiritin apioside, liquiritin, ononin, and glycyrrhizin in hot-water extracts were performed using Tukey's test. Regression coefficients and correlations of determination (R^2) were calculated using regression analysis. Difference were considered significant for p values of < 0.05, $p < 0.01$, or $p < 0.001$, as indicated. Multiple comparison and regression analyses were performed using open- source software R (v. 3.5.2; The R Foundation for Statistical Computing).

III. Results

1. Optimization of analytical conditions

Column, mobile phase modifier, and UV detector wavelength were optimized for HPLC analysis of the four marker compounds in the hot-water extracts of *G. uralensis* roots and rhizomes. Separation of four marker compounds in *G. uralensis* extract was performed on a C_{18}-coated silica column (Capcell Pak Mg II; 4.6 ㎜ × 250 ㎜, 5 ㎛; Shiseido). Mobile phase acidification can increase affinity between a stationary phase and compounds by suppressing the ionizations of acidic compounds, and hence can improve peak shape. Thus, trifluoroacetic acid was added in water at 0.1% *v/v* for gradient elution, as previously described[15,16]. The UV wavelengths chosen were 250 ㎚ for ononin and glycyrrhizin and 275 ㎚ for liquiritin apioside and liquiritin (Figure 2).

2. Validation of analytical methods

The correlation coefficients of the calibration curves of marker compounds were ≥ 0.9999 within the

linear range. LODs and LOQs ranges were 0.04-0.19 ㎍/㎖ and 0.13-0.62 ㎍/mL, respectively (Table 1).

The intra- and inter-day precisions of the HPLC methods were 〈 2.5% and 〈 7.0%, respectively (both represented as RSD, Table 2).

The recovery of the marker compounds in the HPLC methods ranged from ere recovered from 99.89% to 114.61% with RSDs of 〈 7.7% (Table 3).

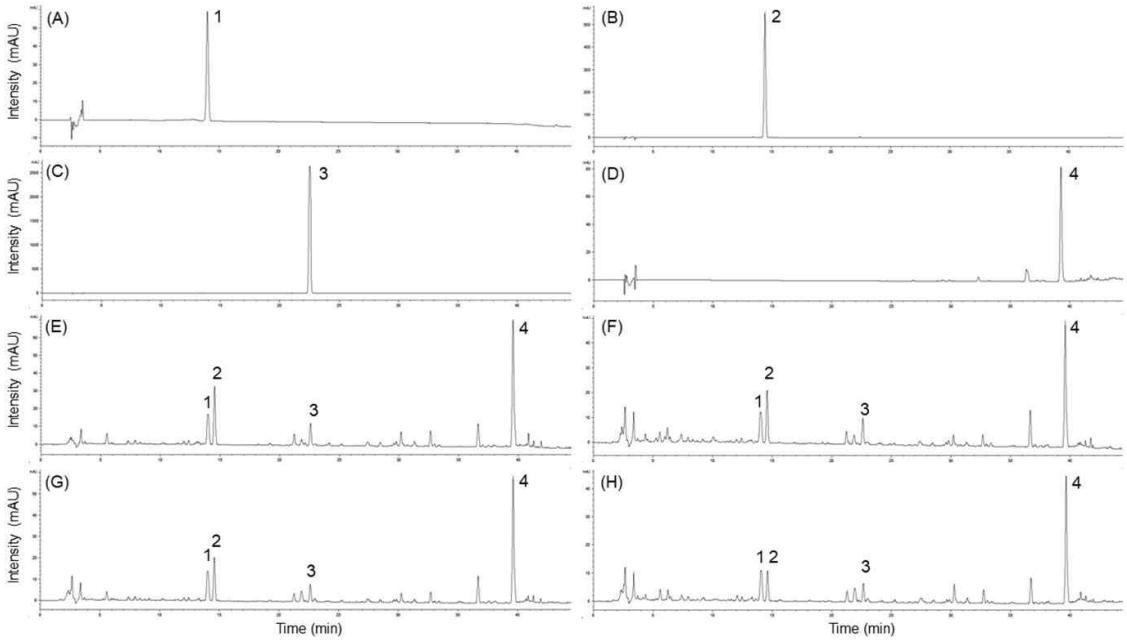

Fig. 2. HPLC chromatograms of the marker compounds (A-D) and hot-water extracts of *G. uralensis* (E), *G. uralensis* + *A. japonica* (F), *G. uralensis* + *A. macrocephala* (G), and *G. uralensis* + *A. chinensis* (H). 1, Liquiritin apioside; 2, liquiritin; 3, ononin; 4, glycyrrhizin.

Table 1. Linear equations, correlation coefficients (r^2), LODs, and LOQs of the four marker compounds

Compound	Regression equation	r^2	Linear range (㎍/㎖)	LOD (㎍/㎖)	LOQ (㎍/㎖)
Liquiritin apioside	y = 15.5395 x + 13.2086	0.9999	3.13-200.00	0.12	0.39
Liquiritin	y = 18.4948 x-10.1753	0.9999	3.13-200.00	0.12	0.39
Ononin	y = 40.2454 x - 2.1201	0.9999	0.31-20.00	0.04	0.13
Glycyrrhizin	y = 3.7757 x-3.1701	0.9999	5.94-380.00	0.19	0.62

LOD, limit of detection; LOQ, limit of quantification; y, peak area (mAU); x, compound concentration (㎍/㎖).

Table 2. Intra- and inter-day precisions of the four marker compounds

Compound	Concentration (㎍/㎖)	Intraday (n = 3)		Interday (n = 3 × 3)	
		RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)
Liquiritin apioside	100.00	1.07	99.91	3.36	101.05
	25.00	2.09	100.09	6.64	98.17
	6.25	0.96	94.92	1.88	94.23
Liquiritin	100.00	1.78	98.15	2.26	100.04
	25.00	1.92	99.73	1.85	100.79
	6.25	0.41	104.54	2.67	107.46
Ononin	10.00	1.36	98.03	1.91	99.88
	2.50	1.08	99.71	1.39	100.59
	0.63	0.65	105.83	1.68	107.38
Glycyrrhizin	190.00	1.33	98.69	1.66	100.23
	47.50	1.24	99.46	1.06	100.01
	11.88	1.18	104.39	1.72	104.98

Conc., concentration; RSD, relative standard deviation (%) = (standard deviation/mean) × 100.

Table 3: Recoveries of the four marker compounds

Compound	Initial conc. (㎍/㎖)	Spiked conc. (㎍/㎖)	Detected conc. (㎍/㎖)	Recovery (%)	RSD (%)
Liquiritin apioside	26.90	2.50	29.43	101.22	4.73
		5.00	32.61	114.20	5.37
		10.00	38.36	114.61	0.59
Liquiritin	12.12	2.50	14.75	105.47	6.75
		5.00	17.34	104.38	3.76
		10.00	22.11	99.89	2.98
Ononin	2.77	0.63	3.40	101.86	7.13
		1.25	4.05	102.96	5.65
		2.50	5.27	100.01	4.50
Glycyrrhizin	108.47	25.00	134.61	104.54	7.67
		50.00	160.51	104.07	3.80
		100.00	210.03	101.56	1.63

Conc., concentration; RSD, relative standard deviation (%) = (standard deviation/mean) × 100.

3. Influence of co-extraction on the extraction efficiencies of the marker compounds

Co-extraction of *G. uralensis* with 20 g of *A. japonica* rhizomes significantly reduced the concentrations of liquiritin apioside (single → combination: 37.037 ± 7.049 ㎍/mL → 29.445 ± 8.156 ㎍/㎖), ononin (2.547 ± 0.558 ㎍/㎖ → 1.872 ± 0.358 ㎍/㎖), and glycyrrhizin (145.796 ± 31.186 ㎍/㎖ → 109.861 ± 12.024 ㎍/㎖). Significant reductions in the concentrations of liquiritin apioside (37.037 ± 7.049 ㎍/㎖ → 30.151 ± 4.190 ㎍/㎖, 29.905 ± 6.864 ㎍/㎖, and 26.522 ± 3.496 ㎍/㎖) and ononin (2.547 ± 0.558 ㎍/㎖ → 1.976 ± 0.457 ㎍/㎖ and 2.000 ± 0.301 ㎍/㎖) occurred in the presence of *A. macrocephala* rhizomes at all weights except 10 g. *A. chinensis* significantly decreased the concentrations of liquiritin apioside (37.037 ± 7.049 ㎍/㎖ → 25.878 ± 5.063 ㎍/㎖, 28.671 ± 4.389 ㎍/㎖, and 29.901 ± 10.667 ㎍/㎖) and ononin (2.547 ± 0.558 ㎍/㎖ → 1.467 ± 0.200 ㎍/㎖, 1.921 ± 0.458 ㎍/㎖, and 2.087 ± 0.693 ㎍/㎖) at all weights and of glycyrrhizin when present at 10 g (145.796 ± 31.186 ㎍/㎖ → 117.794 ± 11.992 ㎍/㎖) (Figure 3).

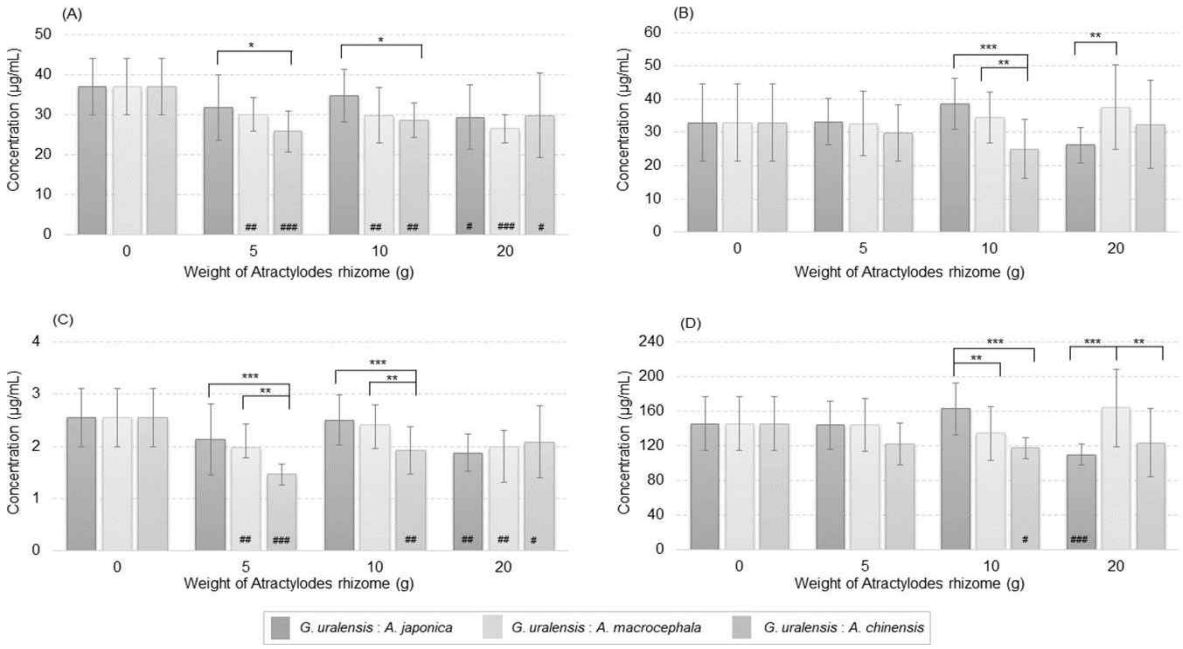

Fig. 3. Change in the concentrations of the four marker compounds after hot water co-extraction of *G. uralensis* (10 g) with each of the three of *Atractylodes* rhizomes (0, 5, 10, and 20 g) for 120 min. A, Liquiritin apioside; B, liquiritin; C, ononin; D, glycyrrhizin.
#, the difference of concentrations between single *G. uralensis* group vs. *G. uralensis* + *Atractylodes* rhizomes group with significance at $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$.
*, the difference of concentrations between *G. uralensis* + *Atractylodes* rhizomes groups with significance at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

As also shown in figure 3, significant decreases in the concentrations of the four compounds were observed when *G. uralensis* roots and rhizomes were extracted with *A. chinensis* (25.878 ± 5.063 ㎍/㎖ at 5 g and 28.671 ± 4.389 ㎍/㎖ at 10 g for liquiritin apioside; 25.039 ± 8.777 ㎍/㎖ at 10 g for liquiritin; 1.467 ± 0.200 ㎍/㎖ at 5 g and 1.921 ± 0.458 ㎍/㎖ at 10 g for ononin; 117.794 ± 11.992 ㎍/㎖ at 10 g for glycyrrhizin) as compared with extractions in the presence of *A. japonica* (31.856 ± 8.193 ㎍/㎖ at 5 g and 34.783 ± 6.637 ㎍/㎖ at 10 g for liquiritin apioside; 38.669 ± 7.621 ㎍/㎖ at 10 g for liquiritin; 2.126 ± 0.682㎍/㎖ at 5 g and 2.507 ± 0.474 ㎍/㎖ at 10 g for ononin; 162.858 ± 29.785 ㎍/㎖ at 10 g for glycyrrhizin) or *A. macrocephala* (34.542 ± 7.637 ㎍/㎖ at 10 g for liquiritin; 1.976 ± 0.457 ㎍/㎖ at 5 g and 2.412 ± 0.387 ㎍/㎖ at 10 g for ononin; 163.903 ± 44.960 ㎍/㎖ at 20 g for glycyrrhizin). Concentrations of liquiritin (26.154 ± 5.360 ㎍/㎖ ↔ 37.533 ± 12.725 ㎍/㎖) and glycyrrhizin (109.861 ± 12.024 ㎍/㎖ ↔ 163.903 ± 44.960 ㎍/㎖) differed significantly at 20 g groups and those of glycyrrhizin (162.858 ± 29.785 ㎍/㎖ ↔ 134.437 ± 30.683 ㎍/㎖) different at 10 g groups, comparing the presences of *A. japonica* and *A. macrocephala* rhizomes.

Concentrations of ononin and glycyrrhizin were significantly reduced by co-extraction with *A. japonica* for 90 min (2.425 ± 0.294 ㎍/㎖ → 1.630 ± 0.516 ㎍/㎖ for ononin; 149.535 ± 19.512 ㎍/㎖ → 118.239 ± 37.466 ㎍/㎖ for glycyrrhizin) and *A. chinensis* (2.425 ± 0.294 ㎍/㎖ → 1.517 ± 0.262 ㎍/㎖ for ononin at 90 min; 107.993 ± 24.315 ㎍/㎖ → 91.283 ± 5.901 ㎍/㎖ for glycyrrhizin at 60 min). Extraction with *A. macrocephala* significantly decreased the concentration of liquiritin at 60 min (27.046 ± 6.948 ㎍/㎖ → 19.037 ± 3.492 ㎍/㎖) and 120 min (38.962 ± 7.025 ㎍/㎖ → 28.539 ± 6.479 ㎍/㎖), and of ononin at 60 min (1.526 ± 0.283 ㎍/㎖ → 1.108 ± 0.334 ㎍/㎖). Concentrations of liquiritin (24.375 ± 6.048 ㎍/㎖ ↔ 19.037 ± 3.492 ㎍/㎖), ononin (1.547 ± 0.512 ㎍/㎖ ↔ 1.108 ± 0.334 ㎍/㎖ at 60 min; 1.630 ± 0.516 ㎍/㎖ ↔ 2.125 ± 0.916 ㎍/㎖ at 90 min), and glycyrrhizin (116.506 ± 22.830 ㎍/㎖ ↔ 93.777 ± 15.137 ㎍/㎖) significantly differed at 60 min and 90 min for extractions in the presence of *A. japonica* or *A. macrocephala*, and co-extraction with *A. chinensis* significantly reduced concentrations of ononin at 90 min (2.125 ± 0.916 ㎍/㎖ → 1.517 ± 0.262 ㎍/㎖) and glycyrrhizin at 60 min (116.506 ± 22.830 ㎍/㎖ → 91.283 ± 5.901 ㎍/㎖), as compared with co-extraction with *A. macrocephala* or *A. japonica* (Figure 4).

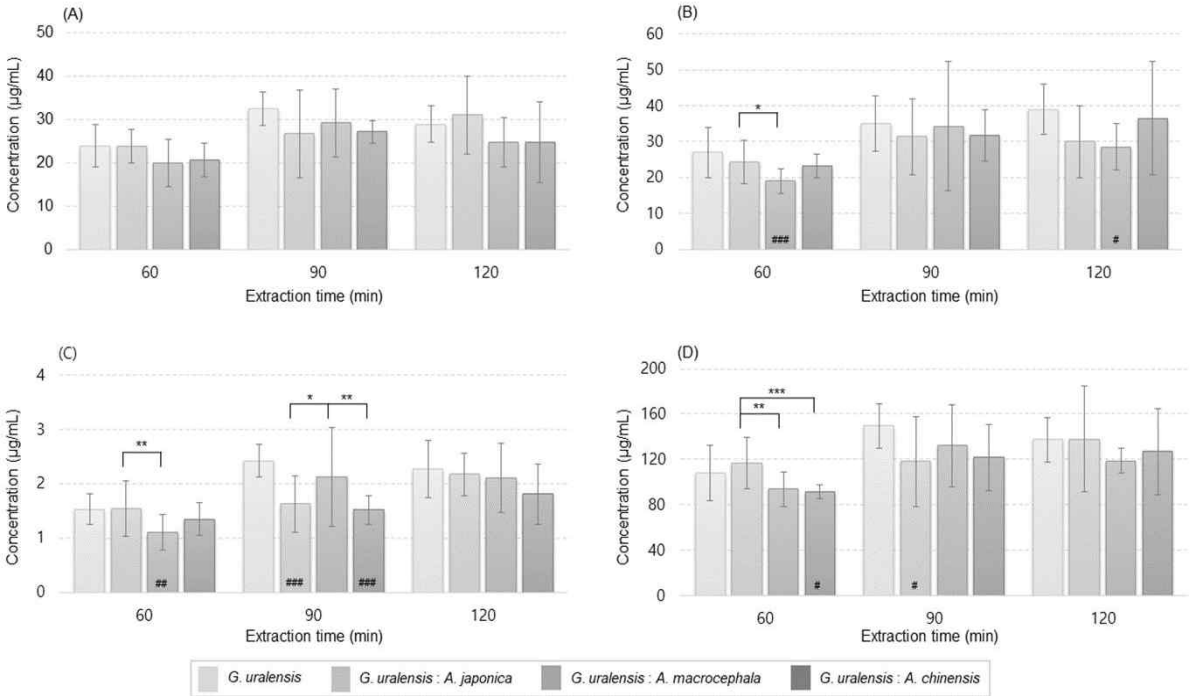

Fig. 4. Change in the concentrations of the four marker compounds after hot water co-extraction of *G. uralensis* (10 g) with *Atractylodes* rhizomes (10 g) for 60, 90, and 120 min. A, Liquiritin apioside; B, liquiritin; C, ononin; D, glycyrrhizin.
#, the difference of concentrations between single *G. uralensis* group vs. *G. uralensis* + *Atractylodes* rhizomes group with significance at $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$.
*, the difference of concentrations between *G. uralensis* + *Atractylodes* rhizomes groups with significance at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

Negative regression coefficients and determination coefficients (R^2)indicated that increasing the weights of *Atractylodes* in co-extraction reduced concentrations of the four marker compounds in *G. uralensis*, with the exception of liquiritin and glycyrrhizin in *G. uralensis* : *A. macrocephala* co-extracts (Figure 5).

In contrast, extraction of *G. uralensis* in the presence of the three *Atractylodes* species time-dependently increased the concentrations of the four marker compounds, as was observed for the extraction of *G. uralensis* alone (Figure 6).

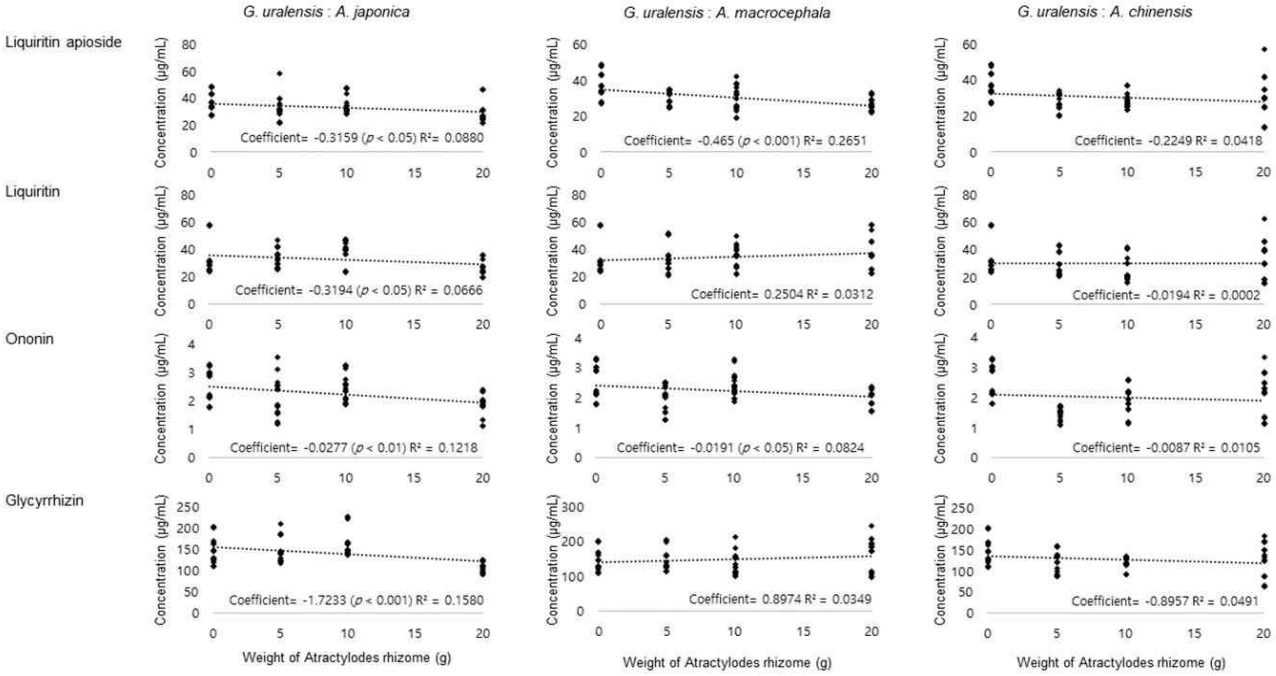

Fig. 5. Regression equations and correlation coefficients (R^2)of the four marker compounds in decoction of *G. uralensis* roots and rhizomes plus *Atractylodes* rhizomes.

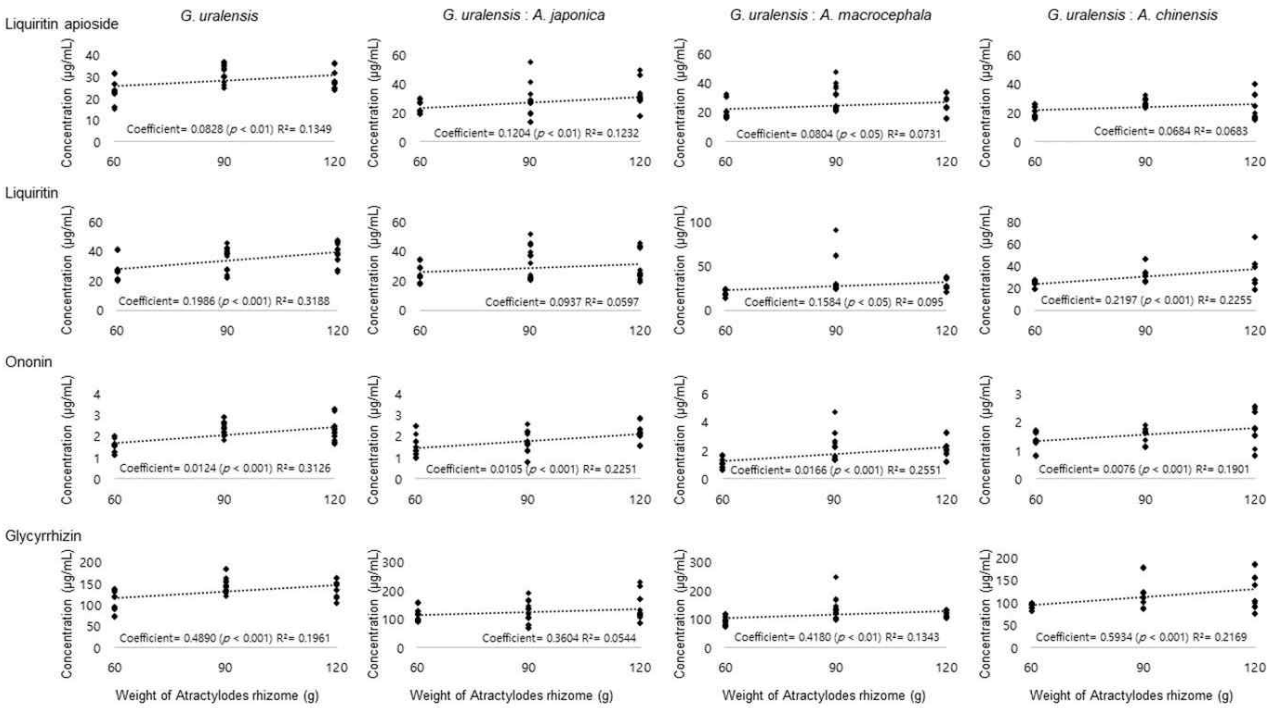

Fig. 6. Regression equations and correlation coefficients (R^2)of the four marker compounds in *G. uralensis* roots and rhizomes (10 g) plus *Atractylodes* rhizomes (10 g) co-extracts in using different decoction times (60, 90, and 120 min).

IV. Discussion

The concentrations of liquiritin apioside, ononin, and glycyrrhizin significantly decreased when *G. uralensis* was co-extracted with the at least one of the three species of *Atractylodes*. This reduction can be explained by possibility that co-extraction with *Atractylodes* rhizomes reduces 1) mass transfers and 2) water-solubilities of *G. uralensis* compounds into hot water, which leads to decrease of extraction efficiencies.

Previous studies have reported that the combination of herbs in decoctions usually reduce the extraction efficiencies of compounds from *G. uralensis*[17-19] and from other herbs[9], and This tendency was also observed in the present study and these reductions were mostly apparent for co-extractions with *A. chinensis*.

Co-extractions with three different *Atractylodes* species for three different times also affected the concentrations of *G. uralensis* marker compounds, except for liquiritin apioside. Moreover, time-dependent increase of the four marker compounds was caused by longer exposure to solvent which usually increases extraction efficiencies of bioactive compounds in extraction process[20,21].

Therefore, in the present study, influence of co-extraction of *G. uralensis* with *Atractylodes* rhizomes and species-specific differences caused by three *Atractylodes* species was observed on the extraction efficiencies of four marker compounds of *G. uralensis*, although these interactions were not confirmed in all co-decoction conditions.

In the viewpoint of herbal decoctions, these results indicate that therapeutic effects of herbal formulae might be affected by species-specific characteristics of the herbal medicines combined because extraction efficiencies of the marker compounds are also altered by herbal combinations.

V. Conclusion

The chemical influences of the co-extraction of the rhizomes of *Atractylodes japonica*, *A. macrocephala*, or *A. chinensis* and *Glycyrrhiza uralensis* roots and rhizomes were evaluated by quantifying liquiritin apioside, liquiritin, ononin, and glycyrrhizin levels using validated HPLC-DAD methods.

1. Co-extraction of *G. uralensis* with *Atractylodes* rhizomes reduced the concentrations of liquiritin apioside, liquiritin, ononin, and glycyrrhizin in *G. uralensis* hot-water extracts and this effect was greatest for co-extraction with *A. chinensis*.

2. The concentrations of liquiritin apioside, liquiritin, ononin, and glycyrrhizin from *G. uralensis* roots and rhizomes were altered by the presence of either of three *Atractylodes* species during hot-water extraction and depended on different weight of *Atractylodes* rhizomes present and extraction times.

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References

1. Shen J, Mo X, Tang Y, Zhang L, Pang H, Qian Y, Chen Y, Tao W, Guo S, Shang E, Zhu S, Ding Y, Guo J, Liu P, Su S, Qian S, Duan JA. Analysis of herb-herb interaction when decocting together by using ultra-high-performance liquid chromatography-tandem mass spectrometry and fuzzy chemical identification strategy with poly-proportion design. J. Chromatogr. A. 2013 ; 1297 : 168-78.
2. Yang Y, Yin XJ, Guo HM, Wang RL, Song R, Tian Y, Zhang ZJ. Identification and comparative analysis of the major chemical constituents in the extracts of single Fuzi herb and Fuzi-Gancao herb-pair by UFLC-IT-TOF/MS. Chin. J. Nat. Medicines. 2014 ; 12(7) : 542-53.
3. Yin G, Cheng X, Tao W, Dong Y, Bian Y, Zang W, Tang D. Comparative analysis of multiple representative components in the herb pair Astragali Radix-Curcumae Rhizoma and its single herbs by UPLC-QQQ-MS. J. Pharm. Biomed. Anal. 2018 ; 148 : 224-29.
4. The Korea Food and Drug Administration, *The Korean Pharmacopeia*, The Korea Food and Drug Administration, 11th edition, Notification No. 2018-68, 2018 : 44, 105.
5. Kim JH, Doh EJ, Lee G. Evaluation of medicinal categorization of *Atractylodes japonica* Koidz. by using internal transcribed spacer sequencing analysis and HPLC fingerprinting combined with statistical tools. Evid.-Based Complementary Altern. Med. 2016 ; 2016 : 1-12.

6. Kim JH, Doh EJ, Lee G. Chemical differentiation of genetically identified *Atractylodes japonica*, *A. macrocephala*, and *A. chinensis* rhizomes using high-performance liquid chromatography with chemometric analysis. Evid.-Based Complementary Altern. Med. 2018 ; 2018 : 1-16.
7. Seo CS, Lee JA, Jung D, Lee HY, Lee JK, Ha H, Lee MY, Shin HK. Simultaneous determination of liquiritin, hesperidin, and glycyrrhizin by HPLC-photodiode array detection and the anti-inflammatory effect of Pyungwi-san. Arch. Pharm. Res. 2011 ; 34(2) : 203-10.
8. Wang Y, He S, Cheng X, Lu Y, Zou Y, Zhang Q. UPLC-Q-TOF-MS/MS fingerprinting of Traditional Chinese Formula SiJunZiTang. J. Pharm. Biomed. Anal. 2013 ; 80 : 24-33.
9. Nose M, Tada M, Kojima R, Nagata K, Hisaka S, Masada S, Homma M, Hakamatsuka T. Comparison of glycyrrhizin content in 25 major kinds of Kampo extracts containing Glycyrrhizae Radix used clinically in Japan. J. Nat. Med. 2017 ; 71(4) : 711-22.
10. Zhang Y, Bo C, Fan Y, An R, Chen L, Zhang Y, Jia Y, Wang X. Qualitative and quantitative determination of Atractylodes rhizome using ultra-performance liquid chromatography coupled with linear ion trap-Orbitrap mass spectrometry with data-dependent processing. Biomed. Chromatogr. 2019 ; 33(3) : e4443.
11. Cho HD, Kim U, Suh JH, Eom HY, Kim J, Lee SG, Choi YS, Han SB. Classification of the medicinal plants of the genus Atractylodes using high-performance liquid chromatography with diode array and tandem mass spectrometry detection combined with multivariate statistical analysis. J. Sep. Sci. 2016 ; 39(7) : 1286-94.
12. Zheng F, Jiang ZB, Zhang X, Hu JP, Li SM, Zhao J, Zeng X. Effect of Glycyrrhizae Radix et Rhizoma combined with Atractylodis Macrocephalae Rhizoma on p53 and p21 gene expression of IEC-6 cells. China J. Chin. Mater. Med. 2015 ; 40(9) : 1798-802. (Chinese)
13. Li G, Nikolic D, van Breemen RB. Identification and chemical standardization of Licorice raw materials and dietary supplements using UHPLC-MS/MS. J. Agric. Food Chem. 2016 ; 64(42) : 8062-70.
14. Wu YP, Meng XS, Bao YR, Wang S, Kang TG. Simultaneous quantitative determination of nine active chemical compositions in traditional Chinese medicine Glycyrrhiza by RP-HPLC with full-time five-wavelength fusion method. Am. J. Chin. Med. 2013 ; 41(1) : 211-9.
15. Basar N, Talukdar AD, Nahar L, Stafford A, Kushiev H, Kan A, Sarker SD. A simple semi-preparative reversed-phase HPLC/PDA method for separation and quantification of glycyrrhizin in nine samples of *Glycyrrhiza glabra* root collected from different geographical origins. Phytochem. Anal. 2014 ; 25(5) : 399-404.
16. Kim JH. Extraction time and temperature affect the extraction efficiencies of coumarin and phenylpropanoids from *Cinnamomum cassia* bark using a microwave-assisted extraction method. J. Chromatogr. B. 2017 ; 1063 : 196-203.
17. Kim JH, Shin HK, Seo CS. Chemical interaction between *Paeonia lactiflora* and *Glycyrrhiza uralensis*, the components of Jakyakgamcho-tang, using a validated high-performance liquid chromatography method: Herbal combination and chemical interaction in a decoction. J. Sep. Sci. 2014 ; 37(19) : 2704-15.
18. Kim JH, Ha WR, Park JH, Lee G, Choi G, Lee SH, Kim YS. Influence of herbal combinations on the extraction efficiencies ofchemical compounds from *Cinnamomum cassia*, *Paeonia lactiflora*, and *Glycyrrhiza uralensis*, the herbal components of Gyeji-tang, evaluated by HPLC method. J. Pharm. Biomed. Anal. 2016 ; 129 : 50-9.
19. Li Z, Liu T, Liao J, Ai N, Fan X, Cheng Y. Deciphering chemical interactions between Glycyrrhizae Radix and Coptidis Rhizoma by liquid chromatography with transformed multiple reaction monitoring mass spectrometry. J. Sep. Sci. 2017 ; 40(6) : 1254-65.
20. Tian M, Yan H, Row KH. Extraction of glycyrrhizic acid and glabridin from Licorice. Int. J. Mol. Sci. 2008 ; 9(4) : 571-7.
21. Liao J, Qu B, Zheng N. Extraction of glycyrrhizic acid from *Glycyrrhiza uralensis* using ultrasound and its process extraction model. Appl. Sci. 2016 ; 6(11) : 319.