대한한의학 방제학회지 제22권 제2호
Herbal Formula Science (HFS)
2014;22(2):15~24
pISSN 1229-1218, eISSN 2288-5641 http://dx.doi.org/10.14374/HFS.2014.22.2.015
Official Journal of The Korean Medicine Society For The Herbal Formula Study
Available at http://www.ompak.okdanche.com HFS

Original Article / 원저

Investigation of difference of Gwakhyangjeonggi-san decoctions produced by different pressure levels and various extraction times

Jung-Hoon Kim[1,2] · Nari Lee[1] · Hyeun-Kyoo Shin[1] · Chang-Seob Seo[1*]

[1]Herbal Medicine Formulation Research Group, Korea Institute of Oriental Medicine, Daejeon, 305-811, Republic of Korea
[2]Division of Pharmacology, School of Korean Medicine, Pusan National University. Yangsan, Gyeongnam 626-870, Republic of Korea

전탕 압력과 전탕 시간의 차이에 따른 곽향정기산 전탕액 비교

김정훈[1,2] · 이나리[1] · 신현규[1] · 서창섭[1*]

[1]한국한의학연구원 한약방제연구그룹
[2]부산대학교 한의학전문대학원 약물의학부

ABSTRACT

Objectives : Gwakhyangjeonggi-san (GJS) which consists of 13 herbal medicines has been used to treat gastrointestinal disorders caused by common cold. This study was performed to compare GJS decoctions produced using different pressure levels for various extraction times.

Methods : Decoctions were prepared by the pressure levels of 0 kgf/cm^2 (non-pressurized) or 1 kgf/cm^2 (pressurized) for 30–180 min. The extraction yield, total soluble solid content (TSSC), and hydrogen ion concentration (pH) were measured, and the contents of the nine marker compounds were determined using high performance liquid chromatography.

Results : The higher pressure and longer extraction time significantly increased TSSC value, while decreased the pH value. However, only extraction time affected the extraction yield of pressurized decoction. Variation of the amounts of chemical compounds was shown in pressurized and non-pressurized decoctions during extraction time. The result of regression analysis showed that pressure and extraction time can influence

to extraction yield, TSSC, pH, and the content of chemical compounds.
Conclusions : This study suggests that the pressure and extraction time can significantly affect the extraction efficiency of components from GJS decoctions.

Keyword : Gwakhyangjeonggi-san, pressure, extraction time, regression analysis

Ⅰ. Introduction

Decoction is the extraction process which is prepared by heating herbal materials with solvent, mainly water, therefore, various physicochemical changes can occur during the process. Diverse extraction factors, such as temperature, extraction time, solvent, or pressure, have been known to change the physicochemical characteristics of various components in herbal decoction by affecting extraction efficiency of the components. Among the extraction factors, pressure and extraction time are key factor that can produce the compositional change of herbal decoction.

Gwakhyangjeonggi-san (GJS) consists of 13 herbal medicines, including *Agastache rugosa* (Fisch. et Meyer) O. Kuntze, *Perilla frutescens* var. *crispa* (Thunb.) H. Deane, *Angelica dahurica* Benth. et Hook. f., *Areca catechu* L., *Poria cocos* F. A. Wolf, *Magnolia officinalis* Rehd. et E. H. Wils., *Atractylodes macrocephala* Koidz., *Citrus reticulata* Blanco, *Pinellia ternata* Breit., *Platycodon grandiflorum* A. DC., *Glycyrrhiza uralensis* Fisch., *Ziziphus jujuba* var. *inermis* (Bunge) Rehder, and *Zingiber officinale* Rosc. Pharmacological studies of GJS reported protective effect against gastric injury, anti-oxidant effect, regulation of intestinal motility, treatment of acute diarrhea and diarrhea-predominant irritable bowel syndrome[1-4].

In the present study, the extraction yield, total soluble solids content (TSSC), hydrogen ion concentration (pH), and the content of marker compoud were compared through GJS decoctions prepared by using pressurized (1 kgf/cm^2) or non-pressurized extraction (0 kgf/cm^2) for 30, 60, 90, 120, 150, and 180 min. The quantification of GJS decoction was performed using high performance liquid chromatography coupled with photodiode array detector. The regression analysis was performed to investigate the influence of extraction factors (pressure and extraction time) on extraction variables, such as extraction yield, TSSC, pH, or the content of each chemical compound.

Ⅱ. Materials and Methods

1. Reagents and herbal materials

Analytical grade-methanol, acetonitrile, and water were purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA). Chlorogenic acid and rosmarinic acid were purchased from Sigma-Aldrich (St Louis, MO, USA). Caffeic acid and hesperidin were purchased from Acros Organics (Morris, NJ, USA). Liquiritin were obtained from NPC Biotechnology (Geumsan, Chungnam, Korea). Apigetrin, oxypeucedanin hydrate, and byakangelicin were supplied from Chengdu Biopurify Phytochemicals Ltd (Chengdu, China),

* Corresponding author : Chang-Seob Seo, PhD. Senior researcher, Herbal Medicine Formulation Research Group, Korea Institute of Oriental Medicine, 1672 Yuseongdae-ro, Yuseong-gu, Daejeon, 305-811, South Korea.
· Tel : 82-42-868-9361, Fax : 82-42-864-2120
· E-mail : csseo0914@kiom.re.kr
• Received : August 26, 2014 / Revised : September 24, 2014 / Accepted : November 12, 2014

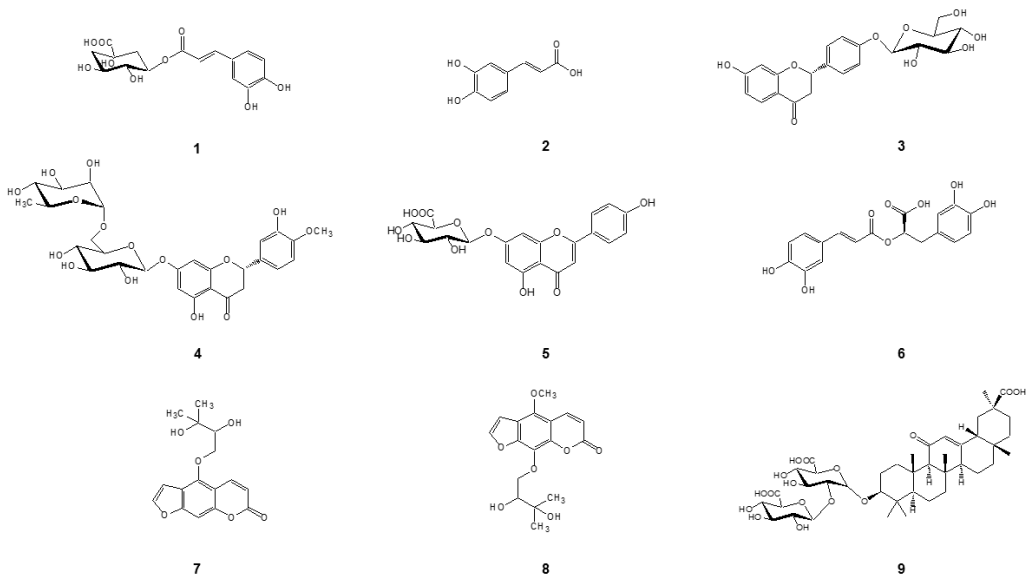

Fig. 1. Chemical structures of 9 standard compounds in Gwakhyangjeonggi-san (GJS).
1: Chlorogenic acid, 2: caffeic acid, 3: liquiritin, 4: hesperidin, 5: apigetrin, 6: rosmarinic acid, 7: oxypeucedanin hydrate, 8: byakangelicin, and 9: glycyrrhizin.

and glycyrrhizin was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). The purity of all of these compounds was > 97% and their chemical structures are shown in Fig. 1.

The herbal medicines were purchased from the herbal medicine company Kwangmyungdang Medicinal Herbs (Ulsan, Korea) (Table 1). A voucher specimen (2014-KE32-1-13) has been deposited in the Herbal Medicine Formulation Research Group of the Korea Institute of Oriental Medicine.

2. Preparation of standard solutions

The standard compounds were accurately weighed and dissolved in methanol to produce stock solutions. Each stock solution was diluted to make working solutions, which were used to construct calibration curves.

3. Preparation of GJS decoctions and samples

The herbal mixture of GJS (675 g corresponding to one formula set, 'Je' in Korean) were extracted at 100°C in water using a high-speed vacuum herb extractor (Cosmos 660, Kyungseo Machine, Incheon, Korea) under pressurized (1.0 kgf/cm^2) or non-pressurized (0 kgf/cm^2) methods for 30, 60, 90, 120, 150, and 180 min. The extraction water was regulated to produce the final volume of the decoction around 3800 mL. A 50 mL of each decoction was lyophilized using a freeze-drier (IlshinBioBase, Dongducheon, Korea). Freeze-dried powder was dissolved in HPLC-grade water and then filtered through a 0.2 μm syringe filter (SmartPor®;Woongki Science, Korea) prior to HPLC injection.

4. Measurements of extraction yield, TSSC, and pH

The extraction yield of each decoction was calculated by the weight of each freeze-dried decoction converted to a percentage of the formula used for a single extraction. TSSC (°Brix)

Table 1. Composition of Herbal Medicines of Gwakhyangjeonggi-san (GJS)

Herbal medicine	Original region	Amount (g)
Agastache rugosa (Fisch. et Meyer) O. Kuntze	Andong, Gyeongbuk, Korea	5.63
Perilla frutescens var. *crispa* (Thunb.) H. Deane	Yeongcheon, Gyeongbuk, Korea	3.75
Angelica dahurica Benth. et Hook. f.	Uljin, Gyeongbuk, Korea	1.88
Areca catechu L.	China	1.88
Poria cocos F. A. Wolf	Pyeongchang, Gangwon, Korea	1.88
Magnolia officinalis Rehd. et E. H. Wils.	China	1.88
Atractylodes macrocephala Koidz.	China	1.88
Citrus reticulata Blanco	Jeju, Korea	1.88
Pinellia ternata Breit.	China	1.88
Platycodon grandiflorum A. DC.	Andong, Gyeongbuk, Korea	1.88
Glycyrrhiza uralensis Fisch.	China	1.88
Ziziphus jujuba var. *inermis* (Bunge) Rehder	Yeongcheon, Gyeongbuk, Korea	3.75
Zingiber officinale Rosc.	Ulsan, Korea	3.75

of each decoction was measured using a refractometer (Pal-α; ATAGO, Tokyo, Japan). A pH was measured with a pH meter (672 pH/Ion meter; Metrohm, Switzerland).

5. Chromatographic conditions

The HPLC-PDA system comprised a Shimadzu LC-20A (Shimadzu Corporation, Kyoto, Japan) equipped with a solvent delivery unit (LC-20AT), autosampler (SIL-20AC), column oven (CTO-20A), degasser (DGU-20A_3), and PDA (SPD-M20A). The acquired data were processed using LabSolutions software (Ver. 5.3; Shimadzu, Japan). Separation was performed on a Gemini C_{18}column (4.6 × 250 mm, 5 μm; Phenomenex, Torrance, CA, USA) maintained at 40 °C. The flow rate was 1.0 mL/min and the injection volume was set to 10 mL. Gradient elution of the mobile phase was applied: 5-70% (B) over 0-40 min, 70-100% (B) over 40-50 min, held for 5 min, and then re-equilibrated to 5% until the end of the analysis. The detection wavelength for each compound was screened from 190 nm to 400 nm and optimal wavelength was set according to the maximum absorption wavelengths of the standard compounds (254, 270, 280, 310, 320, and 330 nm). The analytical conditions in the previous work of our laboratory (Kim et al. *Nat Prod Commun.* 2014) were applied to this study.

6. Statistical analyses

Two-tailed *t*-tests and Dunnett's test were conducted for the two-group and the multi-group comparisons using Microsoft Excel (Microsoft, Redmond, WA, USA) and SYSTAT 10 (SPSS Inc., Chicago, IL, USA). Differences were considered significant at $P < 0.05$, $P < 0.01$, or $P < 0.001$. Regression analysis was performed through extraction yields, TSSC, pH, and the amount of each marker compound using open source software 'R (ver. 3.0.2)'.

Ⅲ. Results and discussion

1. Comparison of extraction yield, TSSC, and pH in GJS decoctions

Except for 90 min, the extraction yields were

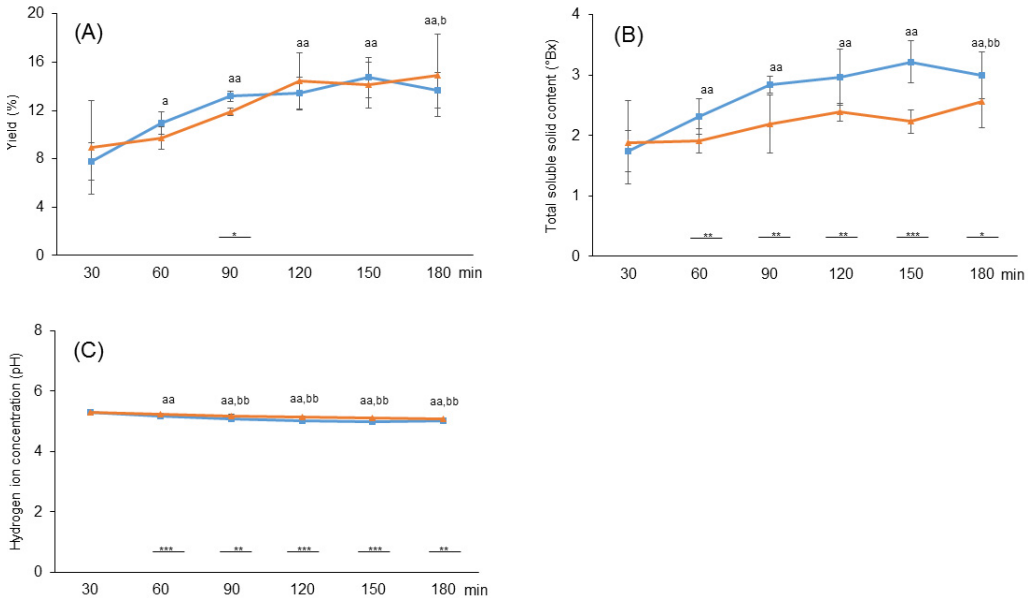

Fig. 2. Variation of extraction yield (A), total soluble solids content (B), and hydrogen ion concentration (C) in GJS decoctions produced by pressurized (■) and non-pressurized (▲) extraction methods for extraction time.
Data expressed as average of triplicate measurements. Statistically significant at $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$ of difference in values between pressurized and non- pressurized extraction methods. $^{a}P < 0.05$, $^{aa}P < 0.01$, and $^{aaa}P < 0.001$ versus the decoction produced by pressurized method at 30 min. $^{b}P < 0.05$, $^{bb}P < 0.01$, and $^{bbb}P < 0.001$ versus the decoction produced by non-pressurized method at 30 min.

not significantly different between both pressurized and non-pressurized decoctions. However, the extraction yield of pressurized decoction was increased with longer extraction time. Unlike the difference between extraction yields of decoctions, TSSC was significantly higher in the decoction produced by pressurized method after initial extraction time, 30 min, and it was increased as extraction time increased. The difference of pH between the pressurized and non-pressurized decoctions was also significant after 30 min, showing that pH of GJS decoction was higher when extracted with non-pressurized method (Fig. 2). This results indicate that higher pressure and longer extraction time can enhance the extraction of ingredients or phenolics from plant cell, or deprotonate the molecules, leading to increased extract weight and lowering pH[5-7].

2. Comparison of the contents of the marker compounds in GJS decoctions

Linear equation, correlation coefficients (r^2), limit of detection, and limit of quantification were applied to this study from previous paper of our laboratory (Kim et al. *Nat Prod Commun.* 2014), shown in Table 2. The nine marker compounds of GJS decoction, such as chlorogenic acid, caffeic acid, liquiritin, hesperidin, apigetrin, rosmarinic acid, oxypeucedanin hydrate, byakangelicin, and glycyrrhizin, were well separated on chromatograms by using the methods described above (Fig. 3).

Chlorogenic acid and caffeic acid were mainly extracted from *P. frutescens* var. *crispa* and rosmarinic acid was extracted from both *A. rugosa* and *P. frutescens* var. *crispa*[8-10]. In the pressurized decoctions, the amounts of chlorogenic acid, caffeic acid, and rosmarinic acid were

Table 2. Linear Equations, Coefficients of Determination (r^2), LOD, and LOQ for the Marker Compounds

Compound	Linear equation	r^2	Linear range (μg/mL)	LOD[a] (μg/mL)	LOQ[b] (μg/mL)
Chlorogenic acid	y = 25348x - 2755.2	0.9999	0.78 - 25.00	0.021	0.072
Caffeic acid	y = 76598x - 623.66	1.0000	0.16 - 5.00	0.007	0.022
Liquiritin	y = 16392.24x - 2609.47	0.9998	0.31 - 40.00	0.030	0.110
Hesperidin	y = 18464.72x - 3003.52	0.9998	0.31 - 40.00	0.030	0.090
Apigetrin	y = 48387x - 239.65	1.0000	0.16 - 5.00	0.010	0.035
Rosmarinic acid	y = 23116.83x + 3596.37	0.9998	0.31 - 40.00	0.030	0.100
Oxypeucedanin hydrate	y = 17270x - 227.06	1.0000	0.78 - 25.00	0.029	0.097
Byakangelicin	y = 20,099.77 x - 27.96	1.0000	0.78 - 25.00	0.024	0.081
Glycyrrhizin	y = 7103.93x - 2718.59	0.9996	0.31 - 40.00	0.070	0.230

[a]LOD, limit of detection; [b]LOQ, limit of quantification.
y, peak area (mAU); x, concentration of compound (μg/mL).
Linear equation, correlation coefficients (r^2), LOD, and LOQ in previous study (Kim et al. *Nat Prod Commun.* 2014) were applied to this study.

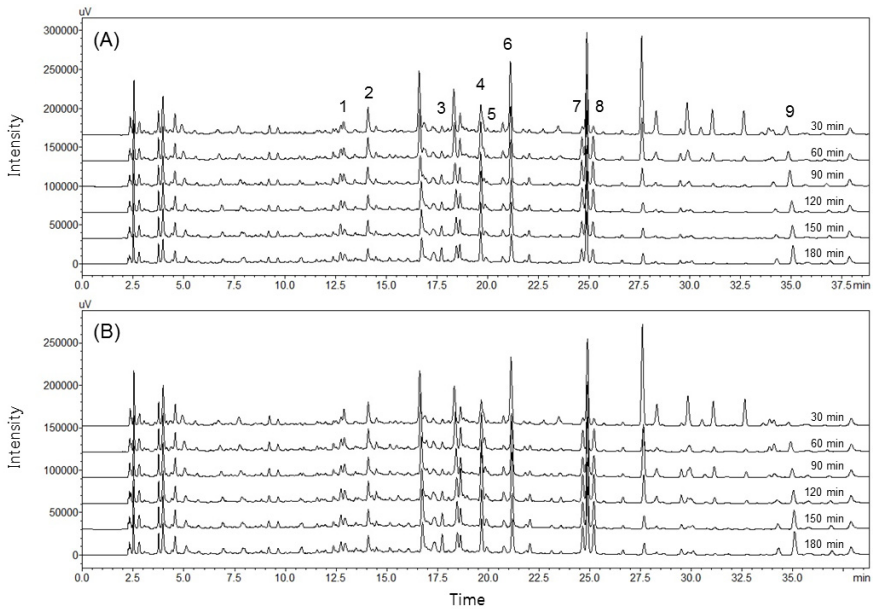

Fig. 3. Representative chromatograms of GJS decoctions produced by pressurized method (A) and non-pressurized method (B) at UV 254 nm.
1: Chlorogenic acid, 2: caffeic acid, 3: liquiritin, 4: hesperidin, 5: apigetrin, 6: rosmarinic acid, 7: oxypeucedanin hydrate, 8: byakangelicin, and 9: glycyrrhizin.

significantly decreased with increasing extraction time compared to initial time, 30 min. However, the amounts of those compounds in non-pressurized decoctions which were also decreased were rather slightly increased after 90 min, showing highest amounts at 120 and 150 min. The amounts of those compounds in the decoction produced by non-pressurized method were higher than the contents of those compounds in pressurized decoctions after 90 min, although the significant

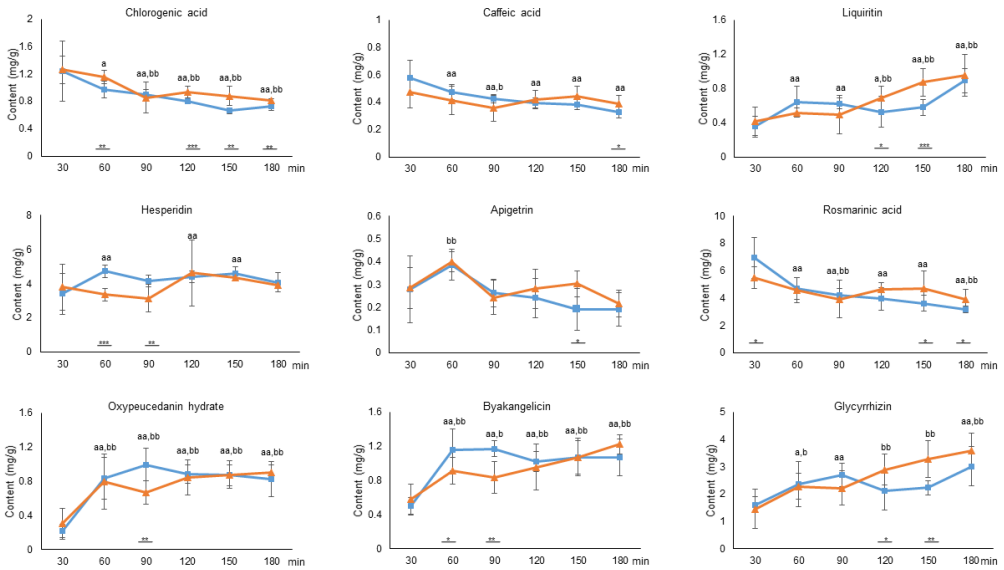

Fig. 4. Variation of the content of 9 marker compounds in GJS decoctions produced by pressurized (■) and non-pressurized (▲) extraction methods for extraction time.
Data expressed as average of triplicate measurements. Statistically significant at $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$ of difference in values between pressurized and non-pressurized extraction methods. $^{a}P < 0.05$, $^{aa}P < 0.01$, and $^{aaa}P < 0.001$ versus the decoction produced by pressurized method at 30 min. $^{b}P < 0.05$, $^{bb}P < 0.01$, and $^{bbb}P < 0.001$ versus the decoction produced by non-pressurized method at 30 min.

difference was shown in some extraction times. The contents of liquiritin and glycyrrhizin, the compounds extracted from *G. uralensis*[11], in non-pressurized decoctions were also significantly higher than the contents of those compounds in pressurized decoctions at 120 and 150 min, however, their contents were increased as extraction time increased. Hesperidin from *C. reticulata*[12] was extracted at higher amount in pressurized decoction before 90 min, however, extraction time did not significantly influence the content variation. The amount of apigetrin, which was the extracted compound from *A. rugosa*[13], was decreased after the peak extraction time, 60 min, both pressurized and non-pressurized decoctions without significance, but the difference between two kinds of decoctions was significant at 150 min. The contents of oxypeucedanin hydrate and byakangelicin, the compounds from *A. dahurica*[14], were significantly increased as extraction time increased showing in non-pressurized decoctions, while the amounts of those compounds in pressurized decoctions were decreased after 90 min when the pressurized extraction method produced significantly higher amounts of two compounds (Fig. 4).

These results indicate that the amounts of chemical compounds from aerial parts of herbal medicines showed decreasing patterns with increasing extract time and non-pressurized method produced higher amounts of those compounds, while the amounts of the compounds from roots generally showed increasing patterns and non-pressurized method also produced higher amounts with longer extraction time.

3. Regression analysis of the influence of the pressure and extraction on extraction yield, TSSC, pH, and the content of each compound

The influence of pressure and extraction time on the variables, such as extraction yield, TSSC,

Table 3. Regression Analysis of Extraction Factors (Pressure and Extraction Time) on Variables

Variable	*p*-values of coefficients		DF	R^2_{adj}	F-value	*p*-value*
	Pressure	Extraction time				
Extraction yield	> 0.05	< 0.001	9	0.7889	21.56	< 0.001
Total soluble solids content	< 0.01	< 0.01	9	0.7484	17.36	< 0.001
Hydrogen ion concentration	< 0.05	< 0.001	9	0.8352	28.87	< 0.001
The content of compound						
Chlorogenic acid	> 0.05	< 0.001	9	0.7859	21.19	< 0.001
Caffeic acid	> 0.05	< 0.05	9	0.4068	4.772	< 0.05
Liquiritin	> 0.05	< 0.001	9	0.6775	12.56	< 0.01
Hesperidin	> 0.05	> 0.05	9	0.1238	1.777	> 0.05
Apigetrin	> 0.05	< 0.05	9	0.3268	3.670	> 0.05
Rosmarinic acid	> 0.05	< 0.01	9	0.0212	6.092	< 0.05
Oxypeucedanin hydrate	> 0.05	< 0.05	9	0.0597	3.918	> 0.05
Byakangelicin	> 0.05	< 0.05	9	0.4126	4.864	< 0.05
Glycyrrhizin	> 0.05	< 0.01	9	0.6477	11.11	< 0.01

DF, Degrees of freedom.
*Statistically significant at $p < 0.05$, $p < 0.01$, or $p < 0.001$.

pH, and the content of each compound, was investigated by regression analysis. Pressure and extraction time significantly influenced the change of extraction yield, TSSC, and pH values, except for pressure on extraction yield. The adjusted regression coefficients (R^2_{adj}) of those three variables were > 0.7 with significant *F*- and *p*-values. The contents of 9 marker compound was not affected by pressure, but significantly affected by extraction time, except for hesperidin. The R^2_{adj} of the contents of 9 marker compounds were $0.02 < r^2 < 0.8$ with significant *p*-values, excluding hesperidin, apigetrin, and oxypeucedanin hydrate with $p > 0.05$ (Table 3). These results demonstrate that higher pressure and longer extraction time can predictably influence to the extraction of ingredients from plant cell, mainly by increasing the extraction, however, those conditions have various effect on the extraction of chemical marker compounds, because individual chemical compounds have their characteristic response to pressure and extraction time[15-17].

Ⅳ. Conclusions

In the present study, we compared Gwakhyangjeonggi-san (GJS) decoctions produced using different pressure levels (0 and 1 kgf/cm^2) for 30-180 min to investigate chemical changes of constituents.

1. A longer extraction time, not pressure, positively affected the extract yield of GJS decoction.

2. Higher pressure and longer extraction time significantly influenced total soluble solid content (TSSC) and the hydrogen ion concentration (pH) of GJS decoction.

3. Various patterns were found in the contents of chemical compounds in different pressure levels and extraction times.

We conclude that the extraction efficiency of the components from GJS was influenced by pressure and extraction time.

Acknowledgements

This work was supported by a grant from the Korea Institute of Oriental Medicine (K14030).

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