

## A Study about Quality Control of Herb Medicine Extract Granules – About DanggwiSayeuggaosaenggangtang(DSGOST)

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**Objectives:** This study investigated quality among three herb medicine extract granules(DSGOST) which were made from different companies to check quality control of herb medicine extract granules.

**Methods:** we selected three DSGOST extract granules which were made from different companies. And we experimented extract granules by method from K.P(Korean Pharmacopoeia), K.H.P(Korean Herbal Pharmacopoeia) of KFDA.

**Results:** In qualitative analysis of DSGOST, we indentified *Akebiae Caulis* (木通), *Asari Herba Cum Radix* (細辛), *Evodiae Fructus* (吳茱萸) in three different DSGOST extract granules. In quantitative analysis of DSGOST, Medication A,B,C contained similar content of Paeoniflorin & Glycyrrhizic acid. However Medication B contains especially lowest value of Cinnamic acid & total Decursin.

**Conclusions:** Herb medicine extract granules have different contents of ingredients although those were made by same prescription. And these differences may influence medicinal effect to patients. So we need to make system of quality control with various research of quantitative & qualitative analysis about herb medicine extract granules.

**Key Words** : DSGOST(DanggwiSayeuggaosaenggangtang), herb medicine extract granules, quality control

## Introduction

The life expectancy of human being has been increased with the help of scientific developments and improved living standards. However, the morbidity of chronic diseases is increasing due to an exposure to environmental toxins and aging of population. According to these reasons, potential demand of herb medicine which is known to be experientially effective & safe from side effects is growing up steadily<sup>1,2)</sup>. Complex herb medicine includes various ingredients, and has distinct character depending on collecting period, and area. So it is necessary to establish standardized manufacture process and quality control process for the constant effect, This matter must to be discussed at national and world wide level<sup>3)</sup>.

Some studies were carried out about quality standardization of distributing herb like project 'Research on Standardization of Herbal Medicines' by public institute<sup>4)</sup>, But It was mostly about harmful contents of herb medicine like heavy metal than quantitative analysis about ingredients of herb medicine or was limited to single herb analysis<sup>5)</sup>. Lately, preference of granuled herb medicine is increasing than boiled herb medicine on account of taking convenience, transportability, storage safety and so on<sup>2)</sup>. However, some complex herb extract granules received revocation of permission because it got poor laboratory results about quality from KFDA inspection in 2014<sup>6)</sup>. In conclusion, Complex herb medicine may have possibility to various quality depending on manufacturing company. But there is no study of comparing quality about complex herb medicines like extract granules which is supplied in market.

Danggwisayeuggaosuyusaenggangtang(DSGOST) was first reported 'Sanghanron(傷寒論)' at East-Han country period<sup>7)</sup>. It is medicine which was added *Angelicae Gigantis Radix* (當歸), *Asari Herba Cum Radix* (細辛) *Evodiae Fructus* (吳茱萸) *Akebiae*

*Caulis* (木通) in Guizhi-tang (桂枝湯) and has heating & warming function to Body system, healing capacity to patient who had Cold Sensitivity of Hands and Feet<sup>8)</sup>. However, There were a few advanced research about effectiveness of DSGOST oral administration in Korea. There was 1 clinical case study of Tal-juh(脫疽)<sup>9)</sup> and 3 experimental studies of through using DSGOST<sup>10-2)</sup>.

Prior to clinical research of DSGOST, we found necessity of research about herb medicine quality control especially using DSGOST. To control quality of herb medicine in market, we selected three complex herb extract granule(DSGOST) which is composed of same prescription and made from different company. And we experimented herb medicine by method from K.P(Korean Pharmacopoeia), K.H.P(Korean Herbal Pharmacopoeia) and announcement of KFDA.

## Materials & Experiment equipment

### 1. Composition of DSGOST (Danggwisayeuggaosuyusaenggangtang)

**Table 1.** Composition of DSGOST  
(Danggwisayeuggaosuyusaenggangtang) in single unit dose

Name	Amount
<i>Angelicae Gigantis Radix</i> (當歸)	1.00g
<i>Cinnamomi Ramulus</i> (桂枝)	1.00g
<i>Paeoniae Radix</i> (芍藥)	1.00g
<i>Akebiae Caulis</i> (木通)	1.00g
<i>Asari Herba Cum Radix</i> (細辛)	0.67g
<i>Glycyrrhizae Radix</i> (甘草)	0.67g
<i>Zizyphi Fructus</i> (大棗)	1.67g
<i>Evodiae Fructus</i> (吳茱萸)	0.67g
<i>Zingiberis Rhizoma Recens</i> (生薑)	1.33g
Total	9.01g

### 2. DSGOST extract granules

We prepared three different DSGOST extract granules which were made from different companies.

We selected the most popular three companies, and named them Medication A, B, C.

### 3. Experiment equipment

#### 1) Identification

Electronic scale(PB303-S), constant-temperature water bath(SH-GLOB22), rotary reflux condenser (N-1), TLC photography system(CAMAG-REPROSTAR2)

#### 2) Quantification

Electronic scale(205TDF), constant-temperature water bath(SH-GLOB22), rotary reflux condenser (N-1), liquid chromatographer(HP1200)

## Methods

### 1. Test of Identification

We experimented three DSGOST extract granules through Thin-Layer-Chromatography(TLC) methods.

#### 1) *Akebiae Caulis* (木通)

##### ① Test liquid of *Akebiae Caulis*

Measure mass of DSGOST as *Akebiae Caulis* 1g and put ethanol 50ml. After attaching reflux condenser, reflux extract during 1 hour and cool off to make 5ml liquid through filtering and evaporative concentration. Cool off liquid and put ether 20ml. Then collect precipitation and dissolve in ethanol 1ml.

##### ② Standard liquid of *Akebiae Caulis*

Measure *Akebiae Caulis* 1.0g and operate same methods as test liquid.

##### ③ Identification

Prepare test liquid & standard liquid of *Akebiae Caulis*. Using chloroform-ethanol-water mixed liquid (13:7:2) as spread solvent. And spray liquid of sulfuric acid. Then heat 10 minutes on 105°C (Using constant temperature bath SH-GLOB22).

#### 2) *Asari Herba Cum Radix* (細辛)

##### ① Test liquid of *Asari Herba Cum Radix*

Measure mass of DSGOST as *Asari Herba Cum Radix* 1g and put ether 50ml. After attaching reflux condenser, reflux extract during 30 minutes. Next, cool off and filter and evaporative dry liquid. Then dissolve in ethanol 2ml.

##### ② Standard liquid of *Asari Herba Cum Radix*

*Asari Herba Cum Radix* 1.0g and operate same methods as test liquid.

##### ③ Identification

Prepare test liquid & standard liquid of *Asari Herba Cum Radix*. Using N-hexane-acetyl acid-ethyl mixed liquid (2:1) as spread solvent. And spray liquid of sulfuric acid. Then heat 10 minutes on 105°C (Using constant temperature bath SH-GLOB22) and expose to ultraviolet rays (365nm).

#### 3) *Evodiae Fructus* (吳茱萸)

##### ① Test liquid of *Evodiae Fructus*

Measure mass of DSGOST as *Evodiae Fructus* 1g and mix & shake 10% ammonia solution 8ml & chloroform 50ml. After waiting in a while, Take separated chloroform liquid layer after putting tragacanth powder 1.5g and shake violently. Wash residual substance with chloroform 10ml and filter combined all chloroform layer. Decompressive concentrate remaining liquid and dissolve residual substance in ethanol 2ml.

##### ② Standard liquid of *Evodiae Fructus*

Measure *Evodiae Fructus* 1.0g and operate same methods as test liquid.

##### ③ Identification

Prepare test liquid & standard liquid of *Evodiae Fructus*. Using water·1-butanol-acetyl acid(31) mixed liquid (5:4:1) & Cyclohexane·chloroform·dethylamine mixed liquid (10:10:1) as spread solvent. And expose to ultraviolet rays (365nm).

### 2. Test of quantitative analysis

We experimented three DSGOST extract granules through Liquid Chromatography methods.

1) Cinnamic acid in *Cinnamoni Ramulus*(桂枝)

① Test liquid of *Cinnamoni Ramulus*

Put DSGOST extract granule amount as *Cinnamoni Ramulus* 1g to soxhlet extraction and put ether 50ml and extract 2 times each for 2 hours. Combine all ether layer and wash with water and filter through sodium sulfuric anhydride (thenardite). Then evaporative dry liquid and put methanol 10ml to residual substance.

② Standard liquid of *Cinnamoni Ramulus*

Cinnamic acid standard material (PA-C-038, purity 99.9%) 0.177mg/ml (made in 2015.02.04.).

③ Quantification

On condition of quantitative Analysis(Table 2) with using water-acetonitrile-acetyl acid(100) mixed liquid(74:25:1) as mobile phase, we calculated amount of *Cinnamoni Ramulus* in DSGOST.

2) Paeoniflorin in *Paeoniae Radix* (芍藥)

① Test liquid of *Paeoniae Radix*

Put DSGOST extract granule amount as *Cinnamoni Ramulus* 1g and put methanol 30ml and ultrasonic extract for 1 hours. Then filter and make liquid to 50ml.

② Standard liquid of *Paeoniae Radix*

Paeoniflorin standard material (PA-P-036, purity 99.8%) 0.230mg/ml (made in 2015.06.08.).

③ Quantification

On condition of quantitative Analysis(Table 2)

with using water-acetonitrile-acetyl acid(31) mixed liquid(86:14:1) as mobile phase, we calculated amount of *Paeoniae Radix* in DSGOST.

3) Glycyrrhizic acid in *Glycyrrhizae Radix* (甘草)

① Test liquid of *Glycyrrhizae Radix*

Put DSGOST extract granule amount as *Glycyrrhizae Radix* (甘草) 0.67g & put water 50ml. Attach reflux condenser and reflux extract in bath during 3 hours. Put 3mol/l sulfuric acid 50ml & hydrolysis in bath during 1 hour. Cool off & put chloroform 50ml. And reflux extract in bath during 30 minutes. Then cool off & migrate to separatory hopper. Take chloroform layer & extract chloroform 30ml 3 times repeatedly. Combine all chloroform layer & Filer through sodium sulfuric anhydride (thenardite). evaporatively dry liquid & put methanol to residual substance for 50ml test liquid. And decompressively concentrate liquid. Finally put methanol and dissolve residual subatances to make 50ml test liquid.

② Standard liquid of *Glycyrrhizae Radix*

Glycyrrhizic standard material (PA-G-052, purity 99.7%) 0.639mg/ml (made in 2015.07.15.).

③ Quantification

On condition of quantitative Analysis(Table 2) with using methanol-water-acetyl acid(100) mixed liquid(78:19:3) as mobile phase, we calculated amount of *Glycyrrhizae Radix* in DSGOST.

**Table 2.** Condition of Quantitative Analysis

	<i>Cinnamoni Ramulus</i> (桂枝)	<i>Paeoniae Radix</i> (芍藥)	<i>Glycyrrhizae Radix</i> (甘草)	<i>Angelicae Gigantis Radix</i> (當歸)
ultraviolet Spectrophotometer				
Detector	W.M* 280nm	W.M* 254nm	W.M* 254nm	W.M* 280nm
Column	waters spherisorb □ ODS2 5 $\mu$ m 4.6mm×250mm(PI-2-10)			
Mobile phase	water-acetonitrile-acetyl acid(100) mixed liquid(74:25:1)	water-acetonitrile-acetyl acid(31) mixed liquid(86:14:1)	methanol-water-acetyl acid(100) mixed liquid(78:19:3)	acetonitrile-water mixed liquid(13:12)
Flow rate	1.0ml/min	1.0ml/min	1.0ml/min	0.7ml/min

W.M\*: wavelength measurement

4) Decursin in *Angelicae Gigantis Radix* (當歸)

① Test liquid of *Angelicae Gigantis Radix*

Put DSGOST extract granule amount as *Angelicae Gigantis Radix* 1g & attach reflux condenser, and put methanol 70ml. Extract from reflux condenser during 2 hours and cool off. Filter and decompressive concentrate liquid. Put methanol and dissolve residual substances to make 50ml test liquid.

② Standard liquid of *Angelicae Gigantis Radix*

Decursin standard material (PA-D-023, purity 98.3%) 0.104mg/ml (made in 2015.05.08.).

③ Quantification

On condition of quantitative Analysis(Table 2) with using acetonitril-water mixed liquid(13:12) as mobile phase, we calculated amount of *Angelicae Gigantis Radix* in DSGOST.

5) Calculate content of test material

① Content of Cinnamic acid (μg/g)

$$\text{concentration of standard liquid (mg/ml)} \times \frac{\text{peak area of test liquid}}{\text{peak area of standard liquid}} \times \frac{\text{volume(ml)}}{\text{amount of test material}} \times \frac{1000\mu\text{g}}{1\text{mg}} \times \text{purity of standard liquid}$$

② Content of Paeoniflorin, Glycyrrhizic Acid, Decursin (mg/g)

$$\text{concentration of standard liquid (mg/ml)} \times \frac{\text{peak area of test liquid}}{\text{peak area of standard liquid}} \times \frac{\text{volume(ml)}}{\text{amount of test material(g)}} \times \text{purity of standard liquid}$$

**Result**

1. Test of identification

We verify same R<sub>f</sub> value and color spot in *Akebiae Caulis* (木通), *Asari Herba Cum Radix* (細辛), *Evodiae Fructus* (吳茱萸) through comparison between standard liquid and test liquid(Table 3). So we identified each DSGOST extract granule had *Akebiae Caulis* (木通), *Asari Herba Cum Radix* (細辛), *Evodiae Fructus* (吳茱萸) contents in three different DSGOST extract granules.

2. Test of quantitative analysis

After experiment of quantification, we calculate amounts of contents in DSGOST. (Table 4)

1) Medication A

- ① *Cinnamoni Ramulus*(桂枝) : 1.97μg/g
- ② *Paeoniae Radix* (芍藥) : 1.63mg/g

- ③ *Glycyrrhizae Radix* (甘草) : 1.97mg/g
- ④ *Angelicae Gigantis Radix* (當歸) : 0.75mg/g

2) Medication B

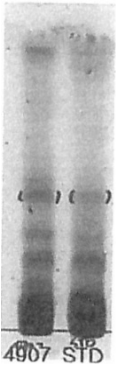
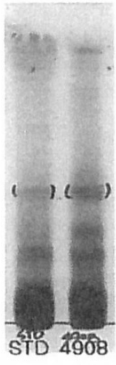

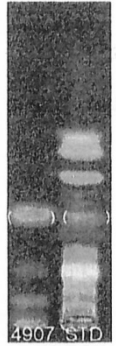
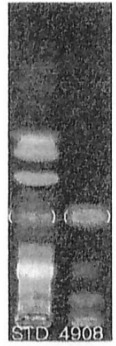
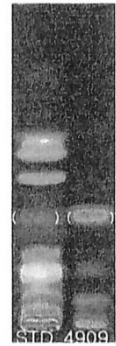
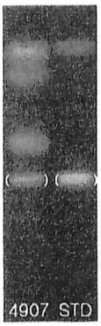

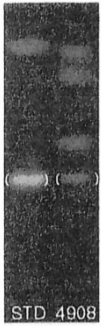

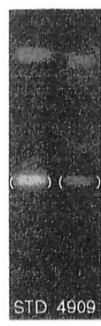

- ① *Cinnamoni Ramulus*(桂枝) : 0.31μg/g
- ② *Paeoniae Radix* (芍藥) : 1.91mg/g
- ③ *Glycyrrhizae Radix* (甘草) : 1.10mg/g
- ④ *Angelicae Gigantis Radix* (當歸) : 0.05mg/g

3) Medication C

- ① *Cinnamoni Ramulus*(桂枝) : 1.84μg/g
- ② *Paeoniae Radix* (芍藥) : 1.30mg/g
- ③ *Glycyrrhizae Radix* (甘草) : 1.14mg/g
- ④ *Angelicae Gigantis Radix* (當歸) : 0.27mg/g

Through results, *Cinnamoni Ramulus* was contained similar level in Medication A, B but it was lowest level in Medication B. *Paeoniae Radix* was contained similar in Medication A, B, C but was slightly more

**Table 3.** Identification among three different DSGOST(DanggwiSayeggaosuyusaenggangtang) extract granules

	Medication A	Medication B	Medication C			
<i>Akebiae Caulis</i> (木通)						
	Brown spot around R <sub>f</sub> 0.5 value both in standard and test liquid					
<i>Asari Herba Cum Radix</i> (細辛)						
	Blue spot around R <sub>f</sub> 0.3 value both in standard and test liquid					
<i>Evodiae Fructus</i> (吳茱萸)						
	① Blue spot around R <sub>f</sub> 0.4 value both in standard and test liquid ② Blue spot around R <sub>f</sub> 0.3 value both in standard and test liquid					

\*Medication A, B, C were DSGOST(DanggwiSayeggaosuyusaenggangtang) extract granules which were made from three different companies.

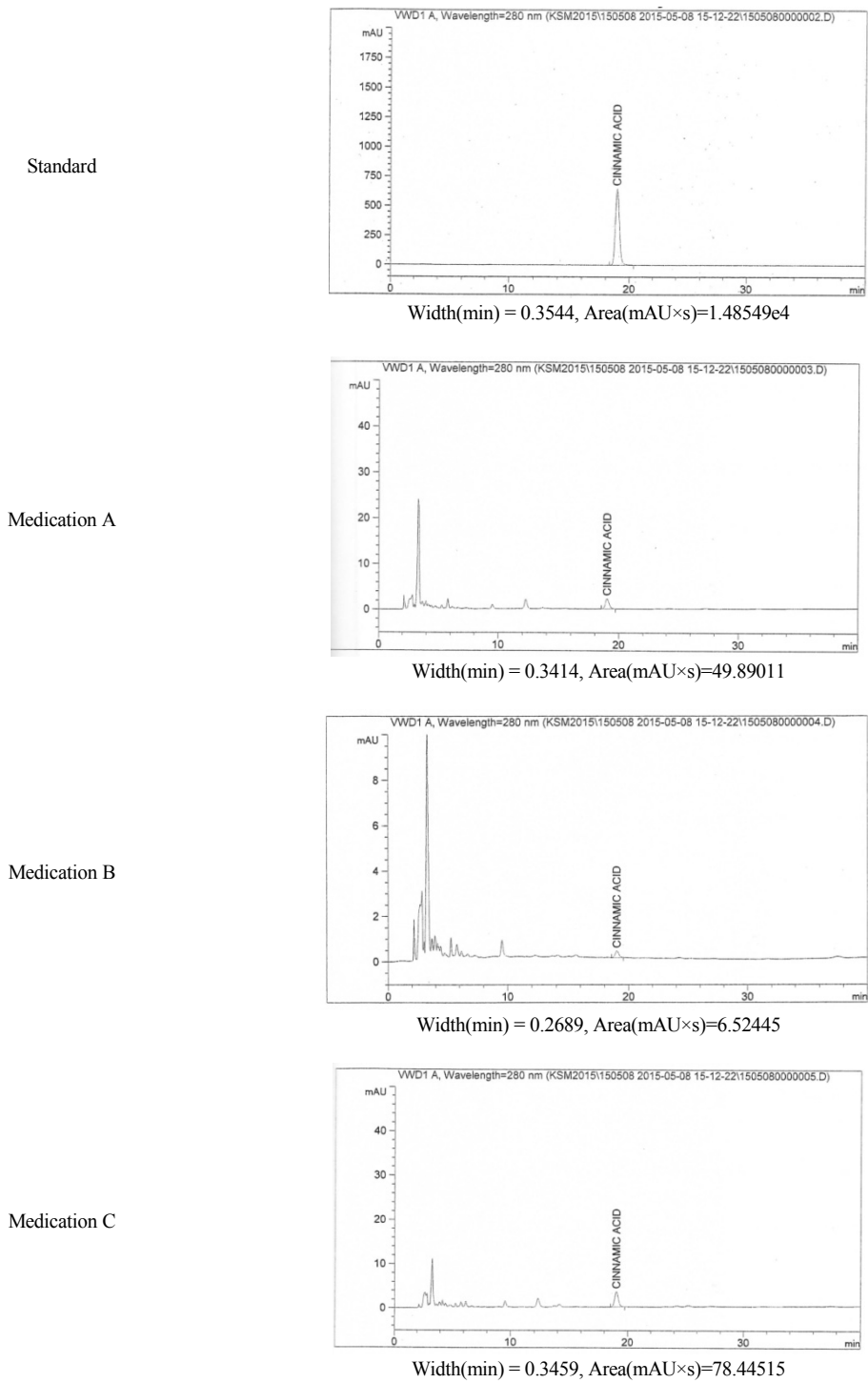
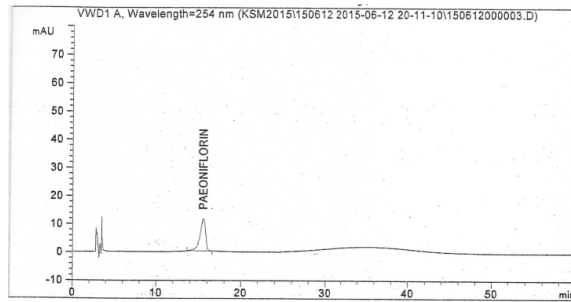


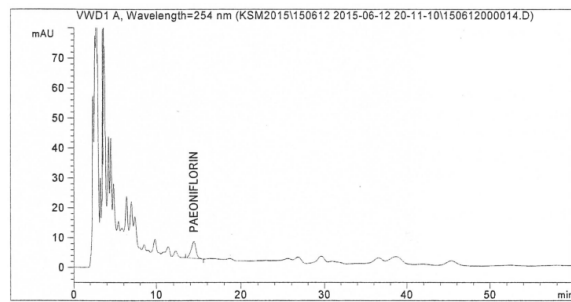
Fig. 1. Quantification of Cinnamic acid(桂枝) among three different DSGOST(DangguiSayeuggaasuyusaenggangtang) extract granules

Standard



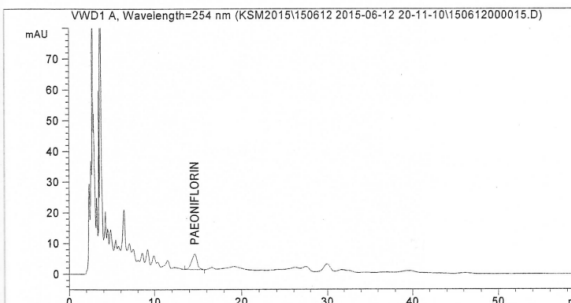
Width(min) = 0.6780, Area(mAU×s)=520.13068

Medication A



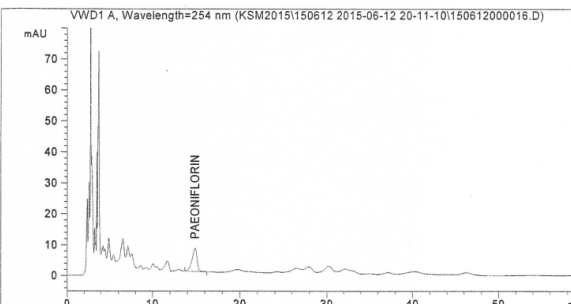
Width(min) = 0.5866, Area(mAU×s)=222.13763

Medication B



Width(min) = 0.6238, Area(mAU×s)=218.09932

Medication C



Width(min) = 0.5999, Area(mAU×s)=296.40384

Fig. 2. Quantification of Paeniflorin(芍藥) among three different DSGOST(Danggwisayeuggaosuysaenggangtang) extract granules



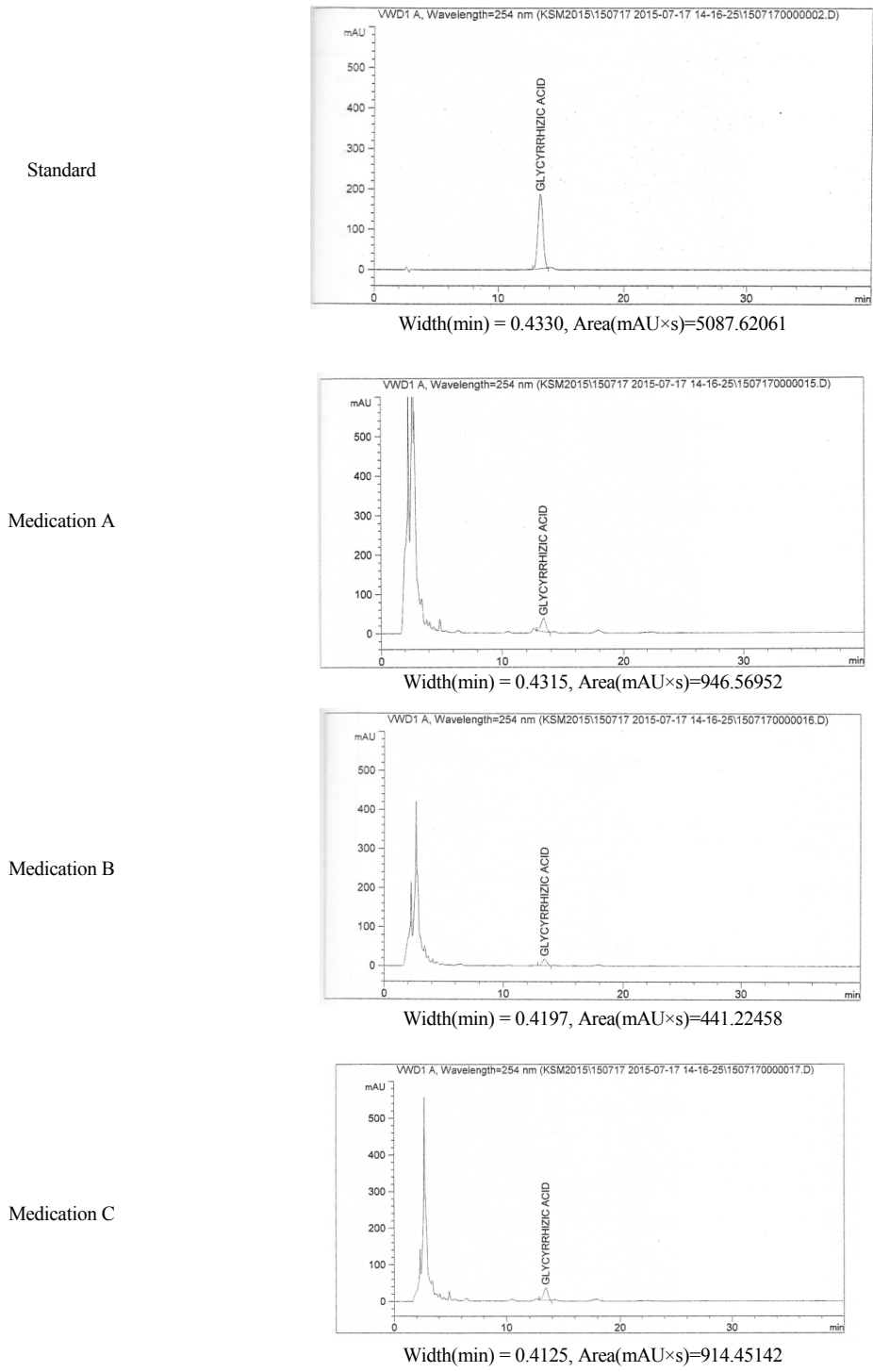


Fig. 3. Quantification of Glycyrrhizic Acid (甘草) among three different DSGOST(DanggwīSayeuggaosuyusaenggangtang) extract granules

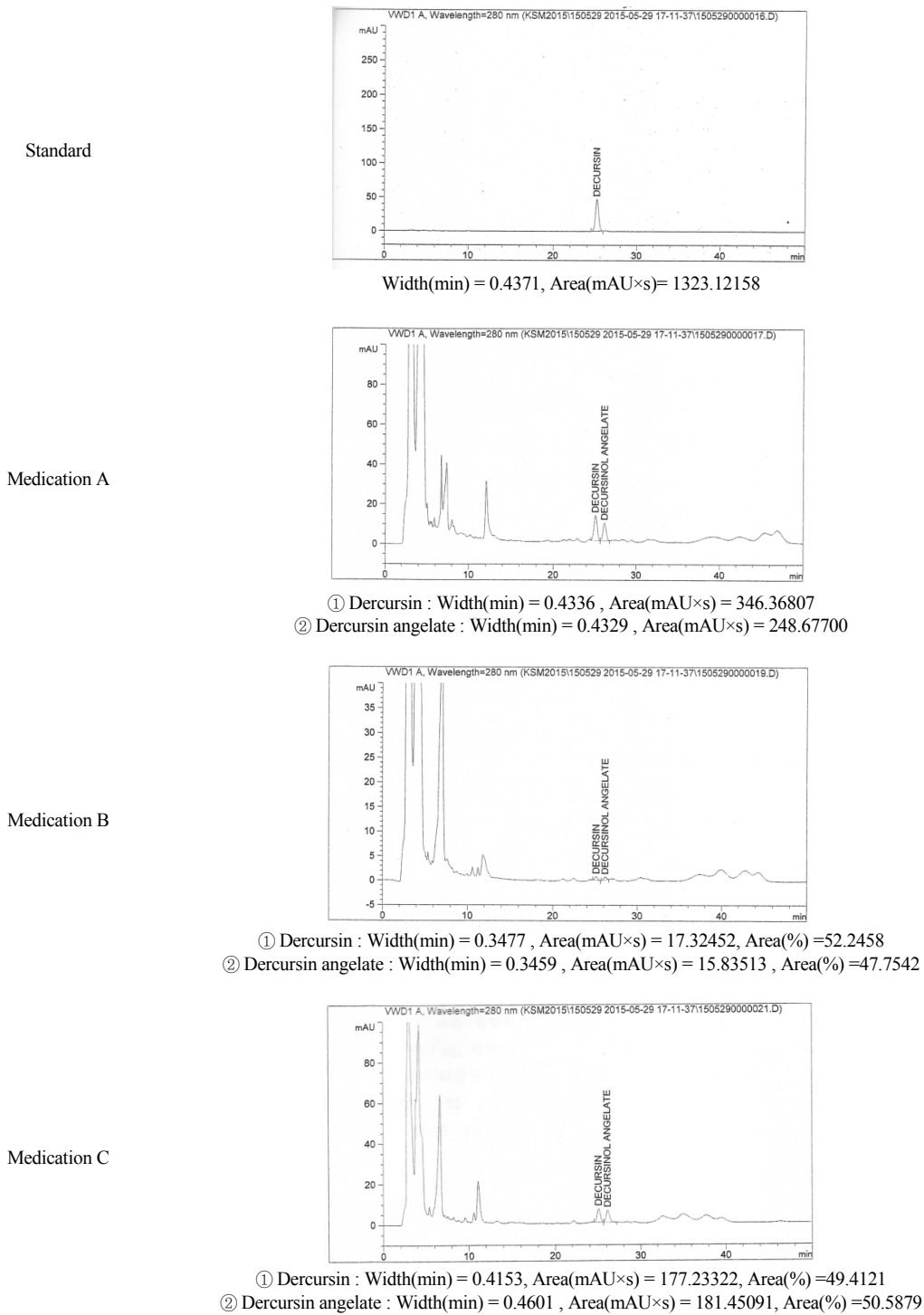


Fig. 4. Quantification of Decursin (當歸) among three different DSGOST(Danggwisayeggaosuyusaenggangtang) extract granules

**Table 4.** Quantitative Analysis about DST(DanggwiSayeuggaosuysaenggangtang)

	Medication A	Medication B	Medication C
Cinnamic acid ( $\mu\text{g/g}$ ) in <i>Cinnamoni Ramulus</i> (桂枝)	1.97	0.31	1.84
Paeoniflorin ( $\text{mg/g}$ ) in <i>Paeoniae Radix</i> (芍藥)	1.63	1.91	1.30
Glycyrrhizic acid ( $\text{mg/g}$ ) in <i>Glycyrrhizae Radix</i> (甘草)	1.97	1.10	1.14
Total Decursin ( $\text{mg/g}$ ) in <i>Angelicae Gigantis Radix</i> (當歸)	0.75	0.05	0.27

higher level in Medication B. *Glycyrrhizae Radix* was contained similar in Medication B, C and was highest level in Medication A. *Angelicae Gigantis Radix* was contained highest level Medication A, medium level in Medication C, lowest level in Medication B.

In conclusion, Medication A was contained with good quality contents. On the other hand, Medication B was contained with poor quality contents comparatively.

## Discussion

Herb medicine is made based on the principle of Korean medicine and composed of various single herb medicine. Demand for the herb medicine is increasing with the prolonged life expectancy and chronic degenerative diseases<sup>2,6</sup>. Herb medicine is known to be experientially effective & safe from side effects<sup>1</sup>. Until now, boiled herb medicines were used more frequently than other forms. But now, however, preference for extract granules is increasing steadily due to its intaking convenience, transportability, and safe storage.

Rules for registering not only single herb medicine product, but also complex herb medicine were made by many countries like 'FDA Guidance for Industry : Botanical Drug Products 2004' in America and 'Traditional Herbal Medicine Products Directive 2004' in Europe. In addition, More than

70% of Gemarn local doctors prescribe natural substance. In Japan and China, Use of Complex herb medicine is increasing as well<sup>6</sup>.

Complex herb medicine includes various ingredients, and has distinct character depending on collecting period, and area. So it is necessary to establish standardized manufacture process and quality control process for the constant effect<sup>3</sup>. Lately, preference for granuled-typed complex herb medicine is increasing. However, a few studies about quality standardization of distributing herb were carried out like project 'Research on Standardization of Herbal Medicines' by public institute<sup>4</sup>, but most of them were focused on harmful contents of herb medicine like heavy metal rather than on quantitative analysis of ingredients, or just limited to a single herb analysis<sup>5</sup>. Conclusively, many herb medicine extract granules might vary in quality depending on which company they were manufactured. However, there is no study comparing qualities of herb medicine extract granules made in different companies.

DanggwiSayeuggaosuysaenggangtang(DSGOST) was first reported in 'Sanghanron(傷寒論)<sup>7</sup>' and it was added *Angelicae Gigantis Radix* (當歸), *Asari Herba Cum Radix* (細辛) *Evodiae Fructus* (吳茱萸) *Akebiae Caulis* (木通) in Guizhi-tang (桂枝湯). This medication has heating & warming function to Human body, healing capacity to patient who have Cold Sensitivity of Hands and Feet. However, there

were a few advanced research about effectiveness of DSGOST oral administration in Korea. There was 1 clinical case study of Tal-juh(脫疽)<sup>9)</sup> and 3 experimental studies of using DSGOST<sup>10-2)</sup>.

Prior to the clinical research of DSGOST, we felt the need to research the quality control of herb medicine especially using DSGOST. So we carried out quality test about three DSGOST extract granules which were distributed to hospital medicine with method by K.P(Korean Pharmacopoeia), K.H.P (Korean Herbal Pharmacopoeia) and announcement of KFDA.

By above results, we indentified *Akebiae Caulis* (木通) through brown spot around  $R_f$  0.5 value, *Asari Herba Cum Radix* (細辛) through blue spot around  $R_f$  0.3 value, *Evodiae Fructus* (吳茱萸) through blue spot around  $R_f$  0.4 value Blue spot around  $R_f$  0.3 value both in standard and test liquid. So we analogize three different DSGOST extract granules include *Akebiae Caulis* (木通), *Asari Herba Cum Radix* (細辛), *Evodiae Fructus* (吳茱萸). But It was simple check like whether specific herb exists or not.

Next, We carried out quantitative analysis of DSGOST. First about Cinnamic acid in *Cinnamoni Ramulus* (桂枝), Medication A(1.97 $\mu$ g/g) & Medication C(1.84 $\mu$ g/g) had high value of Cinnamic acid, But Medication B(1.84 $\mu$ g/g) had lowest value and 1/3 amount of Medication A,C. About Paeoniflorin in *Paeoniae Radix* (芍藥), Medication B(1.91 mg/g) had highest value of Paeoniflorin. Medication A(1.63mg/g) and C(1.30mg/g) had similar value next to Medication B. Next about Glycyrrhizic acid in *Glycyrrhizae Radix* (甘草), Medication A(1.97 mg/g) had highest value of Glycyrrhizic Acid. Medication B(1.10mg/g) and C(1.14mg/g) had similar value next to Medication A. Finally about total Decursin in *Angelicae Gigantis Radix* (當歸) Medication A(0.75 mg/g) had highest value of Total Decursin. Medication C(0.27mg/g) was next level and Medication B(0.05mg/g) was lowest level.

In Conclusion, Medication A,B,C contain similar amounts of Paeoniflorin & Glycyrrhizic acid. However Medication B contains lowest value of Cinnamic acid & total Decursin in particular. So Medication B is believed to have poorer effect compared to A or C. On the other hand, Because Medication A has high value level of contents, Medication A is believed to have the best effect.

As mentioned above, complex herb medicine extract graules made from different companies could have different amounts of ingredients although they were manufactured with the same prescription. And this difference may influence medicinal effect to patients. So we need to make system of quality control by persuing various research of quantitative & qualitative analysis about herb medicine Thereby we could use herb medicine effectively, correctly and safely.

## Conclusion

1. To investigate quality of herb medicine in market, we selected three complex herb extract granules (DSGOST) of the same prescription and made from three different companies.
2. In three different DSGOST, We indentified *Akebiae Caulis* (木通) through brown spot around  $R_f$  0.5 value, *Asari Herba Cum Radix* (細辛) through blue spot around  $R_f$  0.3 value, *Evodiae Fructus* (吳茱萸) through blue spot around  $R_f$  0.4 value Blue spot around  $R_f$  0.3 value both in standard and test liquid. So we analogize that the three different DSGOST extract granules include *Akebiae Caulis* (木通), *Asari Herba Cum Radix* (細辛), *Evodiae Fructus* (吳茱萸).
3. Through quantitative analysis of DSGOST, Medication A,B,C contained similar amounts of Paeoniflorin & Glycyrrhizic acid. However Medication B contains especially lowest value of Cinnamic acid & total Decursin. So Medication B is believed to have poor effect. On the other

hand, Medication is believed to have the best effect since it had highest value level of contents.

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