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Analysis and Comparison of Baicalin, Baicalein and Wogonin Contents in Traditional Decoctions and Commercial Extracts of Scutellariae Radix

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ABSTRACT

Baicalin, baicalein and wogonin are bioactive flavonoids of Scutellariae Radix. In order to compare the contents of baicalin, baicalein and wogonin between traditional decoction and commercial extract, ten crude drugs and ten commercial extracts of Scutellariae Radix were purchased from the market. High performance liquid chromatographic method was used for simultaneous assay of these flavonoid constituents. The separation was performed on an Inertsil ODS-2 column with acetonitrile - 0.005% phosphoric acid (36:64, v/v) as mobile phase at a flow-rate of 1.0 mL/min and detection at 270 nm, and ethyl paraben was used as the internal standard. This method is applicable for the routine quality control of the crude drug and commercial extract of Scutellariae Radix.

The results indicated that the contents of baicalin, baicalein and wogonin in traditional decoctions of each gram of Scutellariae Radix were 62.7 ± 4.6 mg, 5.5 ± 0.2 mg and 1.7 ± 0.1 mg, whereas those in each gram of commercial extracts were 82.3 ± 8.5 mg, 15.0 ± 2.0 mg and 5.3 ± 0.5 mg, respectively.

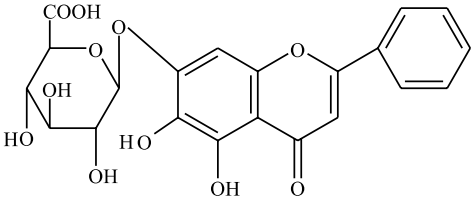
The suggested daily doses of crude drug and commercial extract of Scutellariae Radix are 3.0 – 9.0 g and 0.4 – 9.0 g, respectively. Based on our results, the contents of baicalin, baicalein and wogonin in the maximum daily doses for traditional decoctions of Scutellariae Radix were 564.7 ± 41.7 mg, 49.7 ± 1.9 mg and 15.6 ± 1.0 mg, whereas those for commercial extracts were 241.9 ± 41.1 mg, 53.7 ± 16.4 mg and 17.9 ± 4.6 mg, respectively.

Key words: Scutellariae Radix, baicalin, baicalein, wogonin, decoction, commercial extract

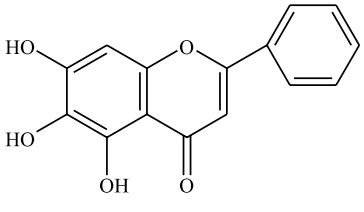
INTRODUCTION

Baicalin, baicalein and wogonin whose structures are shown in Figure 1 are bioactive flavone constituents of Scutellariae Radix. This crude drug is widely used in clinical Chinese medicine as a remedy for inflammation, fever and allergic diseases. In recent studies, baicalin, baicalein, and wogonin have been reported to show anti-inflammatory[(1-4)], anti-allergic[(5)], antioxidant[(6-8)] and anticancer activities[(9,10)]. In addition, baicalin also possessed antiviral activity[(11-13)] and baicalein showed a hypotensive effect[(14,15)].

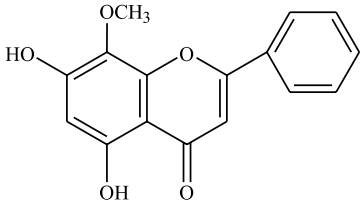
Traditional Chinese medicines are generally administered as decoctions. In recent decades, herbal extract in granule or powder form has been commercialized for clinical use and is the only dosage form of Chinese medicine that is covered by the national health insurance plan in Taiwan. It is important for clinicians to know the contents of these bioactive markers in both traditional decoction and commercial extract in order to decide a proper dosage regimen. This study simultaneously determined baicalin, baicalein and wogonin in both dosage forms of Scutellariae Radix by using an HPLC method with isocratic elution. The object of this study was to analyze and compare their contents in traditional decoctions and commercial extracts based on maximum daily dose.



Baicalin



Baicalein



Wogonin

Figure 1. Structures of baicalin, baicalein and wogonin.

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MATERIALS AND METHODS

I. *Materials and Reagents*

Ten crude drugs of Scutellariae Radix were purchased from ten herbal stores at northern, central and southern Taiwan. Ten brands of commercial extracts of Scutellariae Radix were obtained from herbal stores in Taichung. Baicalin, baicalein and wogonin standards were obtained from Wako (Osaka, Japan). Acetonitrile and methanol (LC grade) were purchased from Mallinckrodt Baker, Inc. (U.S.A.). Phosphoric acid and other reagents used were of analytical grade. Milli-Q plus water (Millipore, Bedford, MA, U.S.A.) was used throughout the study.

II. *Instruments and HPLC Conditions*

The HPLC apparatus was equipped with one pump (LC-6AD, Shimadzu, Japan), an UV spectrophotometric detector (SPD-6A, Shimadzu Japan) and chromatopac (C-R6A, Shimadzu, Japan) with an automatic injector (Series 200 Autosampler, Perkin Elmer, U.S.A.). The Inertsil ODS-2 column (5 μm, 4.6 × 250 mm) was equipped with a guard column (4.6 × 50 mm) (GL Science Inc., Japan). The mobile phase was composed of acetonitrile – 0.005% phosphoric acid (36:64, v/v). The flow-rate was 1.0 mL/min and detection at 270 nm.

III. *Preparations of Standard Solutions*

Baicalin, baicalein and wogonin were accurately weighed and dissolved in methanol to give a series of concentrations in the ranges of 3.1 – 100.0, 2.5 – 80.0 and 2.5 – 80.0 μg/mL, respectively. An equal volume of internal standard solution (ethyl paraben in methanol, 20.0 μg/mL) was added to each standard to afford a final concentration of 10.0 μg/mL. Calibration graphs were plotted by linear regression of the peak-area ratios (drug/internal standard) against concentrations of drugs.

IV. *Preparations of Traditional Decoctions*

Scutellariae Radix was cut into homogeneous pieces and 2.0 g was weighed for each sample. These samples were added to 80 mL of H_2O, heated on a plate heater and boiled for about 2 hr until the volume was slightly less than 20 mL. Each decoction was filtered while hot and hot water was added to make 20 mL. Marc was added to 40 mL of H_2O and heated to boiling again for 1 hr and was concentrated to slightly less than 10 mL. The filtered decoction was added with hot water to make 10 mL. An aliquot of each extract was frozen at -30˚C pending analysis.

V. *Extraction of Commercial Herbal Extracts*

Ten brands of commercial extracts were accurately weighed (0.5 g) and extracted twice with 50 mL 70% MeOH with ultrasonic shaking for 2 hr each time. The extracts were filtered and combined, then sufficient 70% MeOH was added to make 100 mL and frozen at -30˚C pending analysis.

VI. *Assay of Baicalin, Baicalein and Wogonin in Samples*

After thawing, 300 μL decoction sample was mixed with 700 µL methanol and the precipitate was removed by centrifugation at 9860 x g for 15 min. The supernatant was then further properly diluted with methanol. Two hundred µL of the diluted sample was added with 200 µL of ethyl paraben solution (20.0 µg/mL in methanol) and then filtered through 0.45 µm millipore membranes prior to HPLC analysis.

For the samples from commercial extract, proper dilution with methanol was carried out before the addition of internal standard solution. The subsequent procedures were identical to those for decoction samples.

VII. *Validation of Assay Methods*

The system suitability of this method was evaluated by the intra-run and inter-run precision and accuracy of triplicates. The recoveries were further assessed by using the external addition method.

RESULTS AND DISCUSSION

In this study, the water extract was prepared in a different manner from the standard decoction because only 2 g sample was used. In order to extract the constituents more thoroughly, forty times of water was used for extraction and boiled until the volume was reduced to a quarter, which is similar to the usual method of preparing a traditional decoction from 3 bowls to 80% of a bowl. As to the extractions of commercial extracts by using 70% methanol, two times was found almost exhaustive as had been previously determined.

An isocratic HPLC method was used for the simultaneous assay of three flavonoids – baicalin, baicalein, and wogonin of the decoction and commercial extract of Scutellariae Radix. As shown in Figure 2, the three constituents baicalin, baicalein, and wogonin as well as the internal standard (ethyl paraben) were separated effectively within 35 min for both traditional decoction and commercial extract. The retention times of baicalin, baicalein and wogonin were 7.5, 15.6 and 33.9 min, respectively. In contrast to earlier reports on the quantitative analysis of Scutellariae Radix[16,17], the present method used a simple isocratic elution instead of a gradient elution.

Good linear relationships were obtained for baicalin, baicalein and wogonin over the concentration ranges of 3.1 – 100.0 µg/mL, ($y = 0.0425x + 0.0012$, $r = 0.9999$), 2.5 – 80.0 µg/mL, ($y = 0.0623x – 0.0790$, $r = 0.9995$) and 2.5 – 80.0 µg/mL, ($y = 0.0655x + 0.0267$, $r = 0.9996$), respectively. The intra-run and inter-run precision and accuracy analysis of baicalin, baicalein and wogonin indicated that CVs were below 2.6%, 5.3%, 3.8% and relative errors were below 5.3%, 9.7%, 9.7%, respectively. The limits of quantitation (LOQ) were 3.1 µg/mL, 2.5 µg/mL and 2.5 µg/mL for

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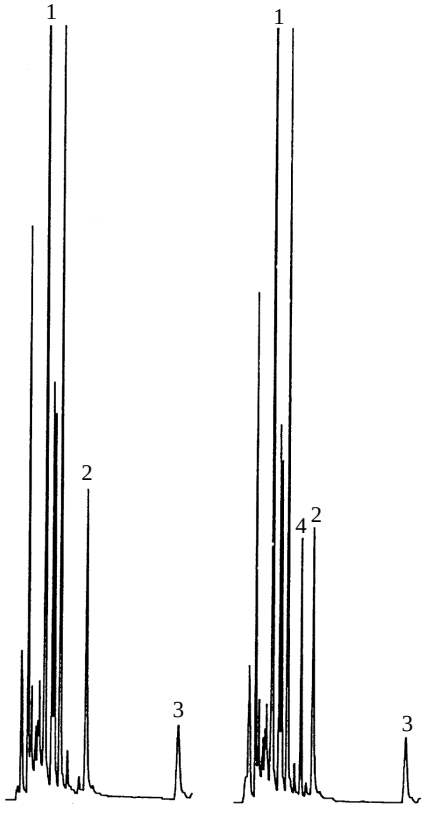

Figure 2. Chromatograms of baicalin, baicalein and wogonin in the water extract of Scutellariae Radix without internal standard (A) and with internal standard (B); 1, baicalin; 2, baicalein; 3, wogonin; 4, internal standard (ethyl paraben).

Table 1. Contents (mg) of baicalin, baicalein and wogonin in water extracts of each gram Scutellariae Radix

Samples	Baicalin	Baicalein	Wogonin
1	79.0 ± 3.1	5.6 ± 0.4	2.0 ± 0.1
2	77.5 ± 2.9	6.2 ± 0.3	2.3 ± 0.2
3	73.0 ± 1.6	6.4 ± 0.2	1.9 ± 0.1
4	70.7 ± 7.2	6.0 ± 0.5	1.6 ± 0.2
5	68.7 ± 3.2	4.8 ± 0.2	1.9 ± 0.1
6	63.0 ± 6.8	5.3 ± 0.1	1.8 ± 0.1
7	59.4 ± 2.9	5.5 ± 0.4	1.8 ± 0.2
8	54.4 ± 4.2	5.8 ± 0.1	1.4 ± 0.1
9	51.1 ± 3.1	5.4 ± 0.3	1.6 ± 0.1
10	30.7 ± 1.3	3.6 ± 0.3	0.9 ± 0.1
Mean ± S.E.	62.7 ± 4.6	5.5 ± 0.2	1.7 ± 0.1
Range	30.7~79.0	3.6~6.4	0.9~2.3

n=3.

baicalin, baicalein and wogonin, respectively. The limits of detection (LOD) were 0.2 μg/mL, 0.3 μg/mL and 0.3 μg/mL for baicalin, baicalein and wogonin, respectively. The recoveries from decoction determined in triplicates were 90.5 ± 0.3% at 12.5 μg/mL, 94.6 ± 2.7% at 25.0 μg/mL, 95.8 ± 1.5% at 50.0 μg/mL for baicalin; 97.9 ± 2.2% at 10.0 μg/mL, 90.7 ± 2.3% at 20.0 μg/mL, 88.5 ± 1.2% at 40.0 μg/mL for baicalein; 110.7 ± 7.2% at 5.0 μg/mL, 99.2 ± 4.5% at 10.0 μg/mL, 96.1 ± 4.3% at 20.0 μg/mL for wogonin, respectively. The recoveries from commercial extracts were 97.8 ± 1.8% at 12.5 μg/mL, 89.2 ± 0.9% at 25.0 μg/mL and 104.3 ± 1.4% at 50.0 μg/mL for baicalin; 87.8 ± 4.2% at 10.0 μg/mL, 110.4 ± 1.8% at 20.0 μg/mL, and 98.2 ± 2.7% at 40.0 μg/mL for baicalein; 103.6 ± 3.0% at 5.0 μg/mL, 99.8 ± 0.5% at 10.0 μg/mL and 97.2 ± 1.0% at 20.0 μg/mL for wogonin, respectively.

Table 2. Contents (mg) of baicalin, baicalein and wogonin in each gram commercial extracts of Scutellariae Radix

Samples	Baicalin	Baicalein	Wogonin
[a]1	123.3 ± 8.1	12.8 ± 0.5	4.8 ± 0.1
[a]2	116.8 ± 8.9	18.9 ± 0.2	6.7 ± 0.1
3	103.7 ± 3.3	13.7 ± 0.0	4.7 ± 0.0
4	93.7 ± 9.9	11.8 ± 0.4	4.7 ± 0.2
[b]5	78.8 ± 1.2	18.2 ± 0.3	6.2 ± 0.1
[a]6	74.7 ± 9.0	13.1 ± 0.6	5.5 ± 0.2
7	71.9 ± 4.9	29.2 ± 4.2	8.0 ± 1.2
8	67.5 ± 7.2	11.8 ± 0.2	5.0 ± 0.1
9	50.5 ± 1.1	15.0 ± 0.7	4.9 ± 0.2
10	41.8 ± 3.4	5.0 ± 0.1	2.2 ± 0.0
Mean ± S.E.	82.3 ± 8.5	15.0 ± 2.0	5.3 ± 0.5
Range	41.8~123.3	5.0~29.2	4.8~8.0

n = 3.

[a]herb powder added as labeled.

[b]herb powder observed.

Table 3. Comparison of the contents (mg) of baicalin, baicalein and wogonin in the maximum daily dose of traditional decoctions and commercial extracts of Scutellariae Radix

Water extracts of crude drug (9 g)			
Samples	Baicalin	Baicalein	Wogonin
1	711.0 ± 22.7	50.2 ± 3.0	18.0 ± 0.9
2	697.5 ± 21.3	58.2 ± 1.9	20.5 ± 1.2
3	657.4 ± 14.7	57.4 ± 2.1	17.0 ± 0.8
4	636.7 ± 65.0	54.4 ± 4.3	14.8 ± 1.9
5	617.9 ± 28.9	43.4 ± 2.1	17.1 ± 0.8
6	567.0 ± 61.3	47.6 ± 1.0	16.5 ± 0.9
7	534.2 ± 21.1	49.5 ± 2.8	16.5 ± 1.3
8	489.9 ± 30.7	49.3 ± 0.4	12.9 ± 0.8
9	459.6 ± 22.6	49.0 ± 2.2	14.0 ± 0.9
10	276.1 ± 9.7	38.1 ± 2.5	8.4 ± 0.4
Mean ± S.E.	564.7 ± 41.7	49.7 ± 1.9	15.6 ± 1.0
Range	276.1~711.0	38.1~58.2	8.4~20.5
Commercial extracts			
Samples (max. daily dose)	Baicalin	Baicalein	Wogonin
[a]1 (1.2 g)	147.9 ± 9.7	15.4 ± 0.5	5.7 ± 0.4
[a]2 (3.6 g)	420.6 ± 32.1	67.9 ± 0.5	24.0 ± 0.6
3 (1.8 g)	186.7 ± 6.0	24.7 ± 0.1	8.5 ± 0.1
4 (2.25 g)	177.3 ± 2.6	41.0 ± 0.6	13.9 ± 0.4
[b]5 (5.4 g)	388.5 ± 26.5	157.8 ± 18.5	43.0 ± 5.2
[a]6 (9.0 g)	454.9 ± 9.7	135.4 ± 5.1	44.2 ± 1.0
7 (2.0 g)	187.4 ± 19.8	23.7 ± 0.8	9.4 ± 0.3
8 (3.6 g)	150.5 ± 12.3	17.9 ± 0.4	7.9 ± 0.1
9 (3.0 g)	224.2 ± 27.1	39.3 ± 1.8	16.6 ± 0.4
10 (1.2 g)	81.0 ± 8.7	14.1 ± 0.2	5.9 ± 0.1
Mean ± S.E.	241.9 ± 41.1	53.7 ± 16.4	17.9 ± 4.6
Range	81.0~454.9	14.1~157.8	5.7~44.2

[a]herb powder added as labeled.

[b]herb powder observed.

Our results indicated the contents of baicalin, baicalein and wogonin in traditional decoctions of each gram Scutellariae Radix were 62.7 ± 4.6 mg, 5.5 ± 0.2 mg and 1.7 ± 0.1 mg, whereas those in each gram commercial extracts

were 82.3 ± 8.5 mg, 15.0 ± 2.0 mg and 5.3 ± 0.5 mg, respectively, as shown in Table 1 and 2.

According to literature[18,19], the suggested daily dose of the crude drug of Scutellariae Radix is in the range of 3.0-9.0 g, whereas that of commercial extract according to the labeling is in the range of 0.4-9.0 g. Therefore, based on our results, the maximum daily doses of baicalin, baicalein and wogonin administered from traditional decoction of Scutellariae Radix are 564.7 ± 41.7 mg, 49.7 ± 1.9 mg and 15.6 ± 1.0 mg, whereas those from commercial extracts are 241.9 ± 41.1 mg, 53.7 ± 16.4 mg and 17.9 ± 4.6 mg, respectively, as shown in Table 3.

When the maximum daily doses of baicalin, baicalein and wogonin are compared among the traditional decoctions of ten crude drugs of Scutellariae Radix, the variations of the three markers are about 2~3 fold, whereas among the ten commercial extracts, the variation of baicalin is about 6 fold, nevertheless, the variations of relatively nonpolar baicalein and wogonin are about 8 ~ 11 fold, indicating larger variation among different brands of commercial extracts than crude drug decoctions.

When the doses of baicalin are compared between traditional decoctions and commercial extracts, the maximum daily dose in commercial extract ranged 81.0~454.9 mg was much lower than that of traditional decoction ranged 276.1~ 711.0 mg. This suggests that the traditional decoction should provide higher efficacy based on the intake of baicalin. As for the maximum daily doses of baicalein and wogonin, three of the ten commercial extracts were found even higher than all the ten traditional decoctions. The labels of two among the three extracts with higher baicalein and wogonin indicated that herb powder was added as excipient and the third extract showed the presence of herb powder under microscope examination, although there was no declaration on the label. The addition of herb powder could account for the higher content of baicalein and wogonin in the commercial extracts.

In order to approach the requirements of GMP as western medicines, the quantitative control of herbal medicinal products is very important. The simultaneous analysis of the three flavonoids presented in this study is applicable for the routine quality control of both the crude drug and commercial extract of Scutellariae Radix. It is suggested for manufacturers to label the contents of active constituents on their herbal products to ensure the quality, efficacy and safety for clinical use.

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黃芩之傳統煎劑與濃縮製劑市場品中黃芩苷、黃芩苷元和漢黃芩素含量之分析與比較

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摘　要

本研究使用等壓高效液相層析法，同時分析黃芩傳統煎劑及濃縮製劑中之黃酮類成分—黃芩苷、黃芩苷元和漢黃芩素之含量。採用Inertsil ODS-2 逆相層析管柱，以acetonitrile – 0.005% phosphoric acid (36:64, v/v) 之混合溶液為移動相，流速為1 mL/min ，檢測波長為270 nm ，並以ethyl paraben為內標準。此分析方法可應用於黃芩藥材和濃縮製劑產品之品質管制。

定量分析各十個市場品之結果顯示，每公克黃芩之傳統煎劑含黃芩苷、黃芩苷元及漢黃芩素平均分別為62.7 ± 4.6 、5.5 ± 0.2 及1.7 ± 0.1 毫克，而濃縮製劑中每公克平均含82.3 ± 8.5 、15.0 ± 2.0 及5.3 ± 0.5 毫克。計算其每日最大劑量，黃芩傳統煎劑平均含黃芩苷、黃芩苷元及漢黃芩素分別為564.7 ± 41.7 、49.7 ± 1.9 及15.6 ± 1.0 毫克，而濃縮製劑則含241.9 ± 41.1 、53.7 ± 16.4 及17.9 ± 4.6 毫克。

關鍵詞：黃芩，黃芩苷，黃芩苷元，漢黃芩素，傳統煎劑，濃縮製劑