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Chemical Influence of *Scutellaria baicalensis*—*Coptis chinensis* Pair on the Extraction Efficiencies of Flavonoids and Alkaloids at Different Extraction Times and Temperatures

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Abstract: The *Scutellaria baicalensis*—*Coptis chinensis* pair is an herbal combination used for the treatment of various heat-related diseases. During the extraction process, two herbs can mutually influence the extraction efficiency of the chemical constituents contained in each herb. The concentrations of five flavonoids from *S. baicalensis* and seven alkaloids from *C. chinensis* were compared in paired or single hot-water extracts at different temperatures (80, 90, and 100 °C) and extraction times (60, 90, and 120 min). Temperature- and time-dependent increases in marker compound concentrations were observed in both paired and single extracts, with the exception of baicalin, berberine, and coptisine in the paired extracts at 100 °C. However, the extractions of the compounds in the paired and single extracts were affected differently by the extraction conditions. Furthermore, the concentrations of most marker compounds in single extracts were 1.09–44.13 times those in paired extracts. The contents of baicalin, wogonoside, coptisine, and berberine, known to be easily aggregated by the flavonoid–alkaloid complex, were changed by 0.024–0.764-fold in the paired extract. The effect of extraction temperature and time on the formation of the flavonoid–alkaloid complex was not significant. The extraction efficiency of the flavonoids and alkaloids can be affected by the pair of *S. baicalensis*—*C. chinensis*, which is a primary factor in the chemical modification of two herb-containing herbal extracts.

Keywords: *Scutellaria baicalensis* roots; *Coptis chinensis* rhizomes; paired extraction; extraction efficiency; extraction condition

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1. Introduction

The roots of *Scutellaria baicalensis* Georgi (Lamiaceae) (SB) and the rhizomes of *Coptis chinensis* Franch. (Ranunculaceae) (CC) have been used to treat heat-induced disorders [1] and are a fundamental pair unit that constitutes diverse traditional herbal formulas [2–5]. The SB–CC pair also exhibits pharmacologically beneficial effects on diabetes mellitus by increasing intestinal disaccharidase activity [6], decreasing toll-like receptor expression [7], and regulating the gut microbiota [8]. Baicalin and berberine, for example, are chemical constituents extracted from the SB and CC and are known to play a significant role in these pharmacological effects [6,9].

The extraction efficiency of the constituents depends on the extracting conditions, such as temperature, time, sample/solvent ratio, and solvent composition. The extraction yields of the constituents are primarily influenced by the temperature, time, and apparatus [10–12]. Previous research on SB or CC has also compared different extraction parameters to maximize extraction yields by extracting methods, moisture content, molar ratio, temperature, and time [13–15].

The compatibility of the SB–CC pair is also a cause for concern, as their combination can alter the chemical or biological properties of a single SB or CC. The SB–CC combination, compared with single herbs, was found to have a more protective effect against ulcerative colitis and higher microbial diversity in the intestine [16]. A synergistic effect of SB–CC complex has also been observed in animal experiments on mice with irritable bowel syndrome that was better than the effects of the sum of berberine and baicalin [17]. Moreover, through proteomic analysis, the administration of SB–CC pair showed differently expressed protein profiling compared to their single herbs [18].

However, studies on how the extract conditions or herbal pair affect the extraction efficiencies of the marker compounds from SB or CC water extracts have not been reported. Nonetheless, it is thought that the same extraction parameters can have a different effect on the constituent extraction efficiencies of single herbs or their herbal pair.

Therefore, the chemical effects of time and temperature on the extraction efficiencies of twelve marker compounds from SB and CC are investigated using high-performance liquid chromatography and multiple regression analysis in both paired extracts of two herbs and their single extracts. Furthermore, the difference in the marker compound yields from each extract of SB or CC are compared to those of their paired extracts to determine the chemical influence of the herbal pair on the properties of the extracted solution.

2. Materials and Methods

2.1. Chemicals and Reagents

Acetonitrile and water (both HPLC grade) were purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA). Chrysin 6-C-arabinoside 8-C-glucoside (1), magnoflorine (2), columbamine (4), epiberberine (5), jatrorrhizine (6), coptisine (7), wogonoside (8), palmatine (9), wogonin (11), and oroxylin A (12) (all purities $\geq$ 98%) were purchased from ChemFaces (Wuhan, Hubei, China). Baicalin (3, 95%), berberine (10, 98%), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The chemical structures of the twelve marker compounds are shown in Figure S1 in the Supplementary File.

The roots of *S. baicalensis* Georgi (Yeosu, Republic of Korea) and the rhizomes of *C. chinensis* Franch. (Sichuan, China) were purchased from Kwangmyungdang Medicinal Herbs (Ulsan, Republic of Korea) and authenticated by the author (J.H. Kim). The voucher specimens (2022-PNUKM-SB01 and CC01) were deposited at the School of Korean Medicine, Pusan National University.

2.2. Sample Preparation

Sliced SB (20 g) and CC (20 g) were extracted separately for the single herbal extraction. The paired herbal extraction was obtained by extracting a total of 40 g of SB and CC with a 1:1 weight ratio. Both groups were extracted with 500 mL distilled water using a heating mantle (MS-DM; Misung Scientific Co., Ltd., Seoul, Republic of Korea) for 60, 90, and 120 min at 80 °C, 90 °C, and 100 °C, respectively. The extracts were cooled to room temperature (20 °C) and were filled up to 500 mL with distilled water. One milliliter of each decoction was centrifuged for 2 min at 13,000 rpm and filtered through a 0.2 μm syringe filter (BioFact, Daejeon, Korea). The filtrate (250 μL) was diluted with 750 μL of distilled water prior to the HPLC analysis.

2.3. Chromatographic Conditions

An Agilent 1200 liquid chromatography system (Agilent Technologies, Palo Alto, CA, USA) with an autosampler, degasser, quaternary solvent pump, and diode array detector (DAD) was used for the quantitative analysis. Chemstation software was used to process the data (ver. B. 04. 03; Agilent Technologies, Inc., Santa Clara, CA, USA). The twelve marker compounds were separated on the ADME column (4.6 mm × 250 mm, 5 μm; Shiseido, Tokyo, Japan) at 30 °C. The flow rate was 1 mL/min, and the injection volume was 10 μL. The mobile phase comprised 0.1% TFA aqueous solution (A) and acetonitrile (B), and the gradient elution was applied as follows: 18–33% (B) for 0–50 min, 33–55%

(B) for 50–60 min, 55–55% (B) for 60–62 min, and then re-equilibrated with 18% (B) until the end of the analysis. The detection wavelengths of the DAD were set as follows: 275 nm for chrysin 6-C-arabinoside 8-C-glucoside, magnoflorine, baicalin, wogonoside, wogonin, and oroxylin A; 345 nm for columbamine, jatrorrhizine, palmatine, and berberine; and 355 nm for epiberberine and coptisine.

2.4. Analytical Method Validation

For the calibration curves of the twelve marker compounds, stock solutions at a concentration of 1000 μg/mL were serially diluted to make seven working solutions. Within the linear ranges of concentrations, the correlation coefficients (r^2) were used to assess the linearity of each calibration curve. The signal-to-noise (S/N) ratios of 3 and 10 were used to determine the limits of detections (LODs) and the limits of quantifications (LOQs).

The repeatability of the analytical methods was determined by analyzing a working solution three times in one day for intraday precision and analyzing it for three days in a row (interday precision). The precisions were expressed as a percentage of the relative standard deviation (RSD): RSD (%) = [(SD/mean) × 100].

The accuracy of the method used was determined by adding known concentrations of marker compounds to the water extracts and calculating the recoveries of the added concentrations as follows: Recovery (%) = [(detected concentration − initial concentration)/spiked concentration] × 100. The results of chromatographic conditioning and method validation are shown in Table S1 in the Supplementary File.

2.5. Statistical Analysis

Tukey's test was used to compare differences in marker compound concentrations between the paired and single extracts, as well as between different extraction conditions (temperatures and times) in each of the paired or single extracts, with significance set at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Multiple regression analysis with two variables (extraction temperatures and times) and a response (the concentrations of the marker compounds) via a second-order polynomial model was used to determine the effects of extraction time and temperature on the concentrations of the marker compounds in both paired and single extracts. With significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, the regression coefficient was used to represent the influence of extraction time and temperature. The estimated second-order polynomial equations of the marker compounds were visualized using a 3-D response surface plot. Tukey's test, multiple regression analysis, and 3-D plots were all performed using the open-source software R (ver. 4.1.2; The R Foundation for Statistical Computing, Vienna, Austria).

3. Results and Discussion

3.1. Differences of the Marker Compound Concentrations by Paired or Single Extraction

Extraction temperature and extraction time are the most influential factors in heat-mediated extraction [12,19]. It was hypothesized that various extraction temperatures and times could affect the extraction efficiencies of the marker compounds between a single herb and herbal pair differently.

The contents of chrysin 6-C-arabinoside 8-C-glucoside increased time-dependently in single extracts at 80 °C, peaking at 90 min and 90 °C. Baicalin content was highest at 120 min and 80 °C and at 90 min and 90 °C, compared to 60 min at each temperature. At 120 min, the contents of columbamine showed significant time-dependent increases at all temperatures, as well as temperature-dependent increases at 60 and 120 min. At 100 °C, epiberberine content was highest at 60 min and at 120 min at both 80 °C and 90 °C. Jatrorrhizine, coptisine, and berberine showed similar temperature-dependent increases in concentrations at 60 and 90 min, and jatrorrhizine and berberine showed maximum levels of concentrations at 120 min compared to 60 min at all temperatures. The contents of wogonoside were mostly proportional to temperature increase, especially at 100 °C,

whereas the contents of palmatine increased in a temperature- and time-dependent manner in all extraction conditions. In all extraction times, both wogonin and oroxylin A showed significantly higher concentrations at 100 °C compared to those at 80 °C, with a significant time-dependent increase at 100 °C (Table 1).

Table 1. The mean concentrations of the marker compounds in the single extracts of *S. baicalensis* or *C. chinensis*.

Compound	Temperature	Time		
		60 Min	**90 Min**	**120 Min**
Chrysin 6-C-arabinoside 8-C-glucoside	80 °C 90 °C 100 °C	40.625 ± 3.428 41.557 ± 1.199 44.022 ± 1.810	46.416 ± 0.736 ^{d} 47.792 ± 1.189 ^{dd} 46.007 ± 1.460	48.320 ± 1.985 ^{eee} 43.521 ± 0.878 46.731 ± 0.724
Magnoflorine	80 °C 90 °C 100 °C	52.783 ± 1.552 51.708 ± 2.354 56.648 ± 3.523	55.370 ± 0.505 54.190 ± 3.166 62.910 ± 4.382 ^{c}	58.735 ± 1.414 60.912 ± 3.405 63.877 ± 3.001
Baicalin	80 °C 90 °C 100 °C	924.320 ± 145.968 950.606 ± 58.115 1077.343 ± 61.543	1088.679 ± 51.385 1143.521 ± 34.794 ^{d} 1180.799 ± 30.917	1126.509 ± 36.244 ^{e} 1033.981 ± 31.679 1200.941 ± 36.866
Columbamine	80 °C 90 °C 100 °C	33.294 ± 0.205 33.575 ± 1.501 38.075 ± 0.228 ^{bb}	36.594 ± 1.144 36.617 ± 1.133 39.750 ± 0.634	38.023 ± 0.905 ^{ee} 40.755 ± 2.342 ^{eee} 45.431 ± 1.896 ^{bbb,cc,eee,ff}
Epiberberine	80 °C 90 °C 100 °C	95.780 ± 0.830 95.480 ± 2.699 109.044 ± 4.300 ^{b,c}	103.358 ± 4.597 104.958 ± 5.612 111.594 ± 2.816	109.247 ± 5.093 ^{e} 113.973 ± 1.507 ^{eee} 113.425 ± 6.051
Jatrorrhizine	80 °C 90 °C 100 °C	21.241 ± 0.615 21.050 ± 0.677 23.559 ± 0.216 ^{bb,cc}	22.939 ± 0.263 23.237 ± 0.700 ^{d} 24.924 ± 0.348 ^{b}	23.720 ± 1.056 ^{ee} 24.740 ± 0.528 ^{eee} 25.771 ± 0.632 ^{b,ee}
Coptisine	80 °C 90 °C 100 °C	105.643 ± 1.240 103.974 ± 3.273 118.958 ± 5.247 ^{b,cc}	114.155 ± 3.274 118.021 ± 2.872 ^{d} 129.212 ± 8.629 ^{bb}	121.410 ± 2.724 ^{ee} 125.534 ± 3.639 ^{eee} 124.364 ± 1.824
Wogonoside	80 °C 90 °C 100 °C	204.398 ± 7.265 214.309 ± 7.757 245.493 ± 12.946 ^{bbb,cc}	228.672 ± 4.270 ^{d} 245.387 ± 6.973 ^{dd} 257.508 ± 3.553 ^{bbb}	236.058 ± 8.624 ^{ee} 240.145 ± 10.003 267.452 ± 10.475 ^{bbb,c}
Palmatine	80 °C 90 °C 100 °C	90.987 ± 0.368 91.047 ± 3.611 101.956 ± 1.603 ^{bbb,ccc}	99.845 ± 1.989 ^{ddd} 100.356 ± 2.184 ^{ddd} 108.150 ± 0.591 ^{bb,cc}	105.568 ± 2.727 ^{eee} 109.630 ± 0.484 ^{eee} 117.262 ± 0.658 ^{bbb,cc,eee,fff}
Berberine	80 °C 90 °C 100 °C	382.550 ± 0.705 380.456 ± 12.640 431.040 ± 19.987 ^{bb,cc}	412.910 ± 9.617 427.644 ± 9.018 ^{dd} 471.017 ± 23.083 ^{bb,c,d}	438.538 ± 2.199 ^{ee} 458.198 ± 17.486 ^{eee} 471.948 ± 5.875 ^{e}
Wogonin	80 °C 90 °C 100 °C	5.422 ± 0.170 6.347 ± 0.214 8.082 ± 0.283 ^{bbb,cc}	6.225 ± 0.091 7.446 ± 0.351 8.450 ± 0.438 ^{bbb}	6.430 ± 0.035 7.687 ± 0.331 ^{e} 10.491 ± 1.133 ^{bbb,ccc,eee,fff}
Oroxylin A	80 °C 90 °C 100 °C	2.221 ± 0.112 2.607 ± 0.110 3.023 ± 0.124 ^{bb}	2.499 ± 0.088 2.768 ± 0.226 3.095 ± 0.107 ^{b}	2.575 ± 0.173 2.941 ± 0.067 3.748 ± 0.405 ^{bbb,cc,ee,ff}

Concentrations were represented as 'mean concentration ± standard deviation'. Difference of the contents in the extract prepared at 80 °C vs. 90 °C, Difference of the contents in the extract prepared at 80 °C vs. 100 °C, with significance at ^{bbb} $p < 0.001$, ^{bb} $p < 0.01$, or ^{b} $p < 0.05$. Difference of the contents in the extract prepared at 90 °C vs. 100 °C, with significance at ^{ccc} $p < 0.001$, ^{cc} $p < 0.01$, or ^{c} $p < 0.05$. Difference of the contents in the extract prepared in 60 min vs. 90 min, with significance at ^{ddd} $p < 0.001$, ^{dd} $p < 0.01$, or ^{d} $p < 0.05$. Difference of the contents in the extract prepared in 60 min vs. 120 min, with significance at ^{eee} $p < 0.001$, ^{ee} $p < 0.01$, or ^{e} $p < 0.05$. Difference of the contents in the extract prepared in 90 min vs. 120 min, with significance at ^{fff} $p < 0.001$, ^{ff} $p < 0.01$, or ^{f} $p < 0.05$.

The concentrations of chrysin 6-C-arabinoside 8-C-glucoside in the paired extracts were significantly proportional to the increase in extraction time at 80 °C and 90 °C, as well as proportional to the increase in temperature at 60 min, with the exception of the highest temperatures at 90 min and 120 min. Magnoflorine was found to have the highest concentrations at 100 °C with various extraction times; jatrorrhizine and wogonoside were highest at 60 and 120 min; palmatine was highest at 90 min and 120 min; and oroxylin A was highest at 60 min. Columbamine and epiberberine concentrations increased proportionally to the increase in extraction time at 80 °C and the increase in temperature at 60 min, respectively. The concentration of wogonin increased in direct proportion to the increase in temperature. The concentrations of coptisine and berberine were significantly proportional to the increase in temperature for all extraction times, with the highest concentrations at 90 min at 80 °C and 90 °C, and were inversely proportional to the increase in extraction time only at 100 °C (Table 2).

Table 2. The mean concentrations of the marker compounds in the paired extracts of *S. baicalensis* and *C. chinensis*.

Compound	Temperature	Time		
		60 Min	**90 Min**	**120 Min**
Chrysin 6-C-arabinoside 8-C-glucoside	80 °C	42.979 ± 1.721	45.801 ± 0.301 ^{d}	46.842 ± 1.089 ^{ee}
	90 °C	43.633 ± 1.219	47.437 ± 0.291 ^{dd}	48.270 ± 0.683 ^{eee}
	100 °C	46.605 ± 0.670 ^{b,cc}	45.272 ± 0.621	44.788 ± 1.185 ^{cc}
Magnoflorine	80 °C	47.083 ± 2.607	50.738 ± 1.846	56.980 ± 2.518
	90 °C	53.791 ± 6.382	59.867 ± 2.934	60.033 ± 3.182
	100 °C	60.148 ± 4.973 ^{b}	67.069 ± 5.333 ^{bb}	70.173 ± 2.892 ^{b}
Baicalin	80 °C	448.536 ± 42.954	458.085 ± 42.282	449.980 ± 17.525
	90 °C	435.675 ± 50.632	479.909 ± 44.931	479.288 ± 18.777
	100 °C	522.052 ± 46.350	462.883 ± 48.275	414.866 ± 5.970
Columbamine	80 °C	10.097 ± 0.360	11.767 ± 0.236 ^{d}	12.470 ± 0.451 ^{eee}
	90 °C	11.169 ± 0.652	10.809 ± 0.726	11.204 ± 0.376
	100 °C	11.184 ± 0.358	11.802 ± 0.559	11.846 ± 0.465
Epiberberine	80 °C	70.078 ± 6.390	80.740 ± 2.475	83.819 ± 2.849
	90 °C	84.766 ± 3.794 ^{a}	84.357 ± 5.561	83.031 ± 6.051
	100 °C	85.078 ± 2.234 ^{b}	93.444 ± 4.014	93.419 ± 8.087
Jatrorrhizine	80 °C	18.344 ± 0.210	19.920 ± 1.948	20.984 ± 1.301
	90 °C	21.024 ± 1.559	22.069 ± 0.937	23.042 ± 1.831
	100 °C	20.785 ± 0.476 ^{b}	23.418 ± 0.999	25.109 ± 1.191 ^{e}
Coptisine	80 °C	6.115 ± 0.212	7.420 ± 0.128 ^{dd,ff}	6.215 ± 0.352
	90 °C	6.170 ± 0.265	8.075 ± 0.286 ^{ddd,fff}	6.615 ± 0.527
	100 °C	10.155 ± 0.316 ^{bbb,ccc,d,eee}	9.187 ± 0.176 ^{bbb,ccc}	8.483 ± 0.216 ^{bbb,ccc}
Wogonoside	80 °C	156.208 ± 4.720	160.761 ± 2.859	161.743 ± 3.883
	90 °C	162.507 ± 2.888	172.477 ± 2.425	170.679 ± 3.829
	100 °C	175.403 ± 1.426 ^{bb}	170.966 ± 8.131	184.174 ± 8.246 ^{bb}
Palmatine	80 °C	73.373 ± 1.975	78.377 ± 4.610	81.476 ± 4.359
	90 °C	80.246 ± 5.047	81.691 ± 2.293	87.902 ± 4.467
	100 °C	79.361 ± 1.092	90.961 ± 5.473 ^{b,d}	95.889 ± 3.575 ^{bb}
Berberine	80 °C	9.744 ± 0.379	11.797 ± 0.222 ^{ddd,ff}	9.937 ± 0.571
	90 °C	11.808 ± 0.403 ^{aaa}	13.058 ± 0.556 ^{a,ddd,fff}	11.128 ± 0.549
	100 °C	15.783 ± 0.474 ^{bbb,ccc,ee}	15.157 ± 0.201 ^{bbb,ccc}	13.984 ± 0.170 ^{bbb,ccc}

Table 2. *Cont.*

Compound	Temperature	Time		
		60 Min	**90 Min**	**120 Min**
Wogonin	80 °C	2.659 ± 0.098	3.016 ± 0.269	2.910 ± 0.120
	90 °C	2.953 ± 0.125	3.198 ± 0.071	3.405 ± 0.376
	100 °C	3.808 ± 0.358 ^{bbb,cc}	3.430 ± 0.240	3.550 ± 0.178
Oroxylin A	80 °C	1.062 ± 0.045	1.185 ± 0.065	1.199 ± 0.047
	90 °C	1.259 ± 0.111	1.219 ± 0.063	1.303 ± 0.101
	100 °C	1.489 ± 0.180 ^{bb}	1.301 ± 0.135	1.297 ± 0.061

Concentrations were represented as 'mean concentration ± standard deviation'. Difference of the contents in the extract prepared at 80 °C vs. 90 °C, with significance at ^{aaa} $p < 0.001$, or ^{a} $p < 0.05$. Difference of the contents in the extract prepared at 80 °C vs. 100 °C, with significance at ^{bbb} $p < 0.001$, ^{bb} $p < 0.01$, or ^{b} $p < 0.05$. Difference of the contents in the extract prepared at 90 °C vs. 100 °C, with significance at ^{ccc} $p < 0.001$, ^{cc} $p < 0.01$. Difference of the contents in the extract prepared in 60 min vs. 90 min, with significance at ^{ddd} $p < 0.001$, ^{dd} $p < 0.01$, or ^{d} $p < 0.05$. Difference of the contents in the extract prepared in 60 min vs. 120 min, with significance at ^{eee} $p < 0.001$, ^{ee} $p < 0.01$, or ^{e} $p < 0.05$. Difference of the contents in the extract prepared in 90 min vs. 120 min, with significance at ^{fff} $p < 0.001$, ^{ff} $p < 0.01$.

Although there were no significant differences in concentrations among the samples, the extraction time and temperature affected the concentrations of the marker compounds in the single extracts proportionally. These findings are consistent with previous reports that showed increasing the extraction temperature to 80–100 °C increased the contents of baicalin and wogonoside in boiled water extracts [20]. However, in the paired extracts, the positive proportionality between contents and conditions was not clearly observed: the temperature mostly affected the contents of the marker compounds positively, whereas the time provided peak contents in the middle of the extraction time for several cases at 80 °C and 90 °C. Moreover, the concentrations of chrysin 6-C-arabinoside 8-C-glucoside, baicalin, coptisine, and berberine showed negative proportionality with the increase in extraction time at the highest extraction temperature.

3.2. Influence of Extraction Conditions on the Marker Compound Extractions

Multiple regression analysis revealed differences in the effects of extraction temperature and time on the extraction of the marker compounds between the paired and single extracts, as shown in Table 3.

Temperature and time significantly affected the concentrations of chrysin 6-C-arabinoside 8-C-glucoside in the paired extracts by the first order, while extraction time significantly affected the concentrations of chrysin 6-C-arabinoside 8-C-glucoside in the single extracts by first and second order. Temperature influenced the concentrations of magnoflorine, jatrorrhizine, and palmatine in single extracts in a negative first order and a positive second order. The extraction times of baicalin and wogonoside in single extracts showed significant influences in the first (positive) and second (negative) orders, and the temperature in the second order positively affected the baicalin extraction. Extraction time was an influential factor in the concentrations of columbamine in the paired extracts in the first order, while the temperature was an influential factor in the single extracts in the second order, negative in the first order and positive in the second order.

Furthermore, both extraction time and temperature in the first and second orders had a significant impact on the concentrations of coptisine and berberine in the paired extracts. In contrast, the extraction time in the first order had a positive impact on two compounds in the single extracts, and the temperature in the second order had a positive impact on berberine.

Moreover, the interactions between the extraction temperature and time on the concentrations were negatively significant to the concentrations of chrysin 6-C-arabinoside b-C-glucoside, baicalin, columbamine, coptisine, and berberine in the paired extracts, as seen in the interaction coefficients (temperature:time) [21]. These findings show that two variables, temperature and time, had a significant inverse relationship with the extraction

efficiencies of those compounds when SB and CC were extracted together [22,23]. Significant interactions between the above two variables were observed as negative on chrysin 6-C-arabinoside b-C-glucoside but positive on wogonoside in single extracts.

Table 3. The coefficients of multiple regression analysis of the marker compounds in the paired or the single extract of *S. baicalensis* and *C. chinensis*.

Compound	Extraction	Regression Coefficient (Significance at *** $p < 0.001$, ** $p < 0.01$, or * $p < 0.05$)						
		Intercept	Temp.	Time	Temp.:Time	Temp.:Temp.	Time:Time	Adjusted r^2
Chrysin 6-C-arabinoside 8-C-glucoside	Pair	−88.5300	2.3610 *	0.5933 ***	−0.0047 ***	−0.0107	−0.0007	0.5352 ***
	Single	66.7608	−1.5171	0.9644 ***	−0.0041 *	0.0106	−0.0029 **	0.5407 ***
Magnoflorine	Pair	36.7900	−0.7422	0.3738	0.0001	0.0080	−0.0013	0.7567 ***
	Single	253.3000 *	−4.8310 *	0.0377	0.0011	0.0278 *	−0.0001	0.6449 ***
Baicalin	Pair	−823.4690	18.8697	9.5140	−0.0905 *	−0.0556	−0.0095	0.0723
	Single	3738.7794	−91.4914	25.2441 **	−0.0655	0.5706 *	−0.0949 **	0.6086 ***
Columbamine	Pair	33.9548	−0.7038	0.1716 *	−0.0014 *	0.0047	−0.0001	0.4264**
	Single	151.7000 **	−2.7230 *	−0.1977	0.0022	0.0155 *	0.0006	0.8629 ***
Epiberberine	Pair	−11.3266	0.3454	1.0811	−0.0045	0.0038	−0.0031	0.5148 ***
	Single	166.7000	−2.978	0.9791	−0.0076	0.0227	−0.0005	0.6690 ***
Jatrorrhizine	Pair	−38.3800	1.1540	−0.0256	0.0014	−0.0062	−0.0003	0.6899 ***
	Single	59.8904 *	−1.1041 *	0.1373	−0.0002	0.0068 *	−0.0004	0.8428 ***
Coptisine	Pair	54.6919 **	−1.4890 **	0.3137 ***	−0.0015 *	0.0098 ***	−0.0010 ***	0.8442 ***
	Single	198.0029	−4.3063	1.7775 **	−0.0086	0.0311	−0.0042	0.7167 ***
Wogonoside	Pair	76.6400	1.2430	−0.1946	0.0027	−0.0035	0.0004	0.6422 ***
	Single	437.9281	−9.55309	3.0117 **	−0.0081	0.0665	−0.0102 *	0.8170 ***
Palmatine	Pair	66.0000	−0.0102	−0.3256	0.0070	−0.0004	−0.0007	0.7169 ***
	Single	327.1000 ***	−6.0480 ***	0.2234	0.0006	0.0362 ***	0.0000	0.9400 ***
Berberine	Pair	28.5085	−0.9508 *	0.3913 ***	−0.0017 **	0.0074**	−0.0014 ***	0.9322 ***
	Single	957.1066	−19.1585	4.1149 *	−0.0126	0.1257 *	−0.0112	0.8258 ***
Wogonin	Pair	−0.2535	−0.0035	0.0407	−0.0004	0.0004	0.0000	0.5820 ***
	Single	30.0000	−0.5978	−0.0859	0.0012 *	0.0036	0.0000	0.8786 ***
Oroxylin A	Pair	−2.048	0.0444	0.0181	−0.0003	0.0000	0.0000	0.4874 **
	Single	8.4160	−0.1441	−0.0330	0.0003	0.0009	0.0001	0.8078 ***

With the exception of baicalin in the paired extracts, the correlation coefficient (r^2) between the concentrations of the marker compounds and two variables (temperature and time), which ranged from 0.4264 to 0.9400 for adjusted r^2, indicates better correlation and prediction among them when it is closer to 1 [24,25]. A 3-D response plot was used to visualize the predicted values of the second-order polynomial equation in the paired and single extracts (Figures 1 and 2).

3.3. Influence of Herbal Pair on the Marker Compound Extractions

All marker compounds showed significantly higher concentrations in single extracts than in paired extracts at most extraction conditions: 1.09 (80 °C, 60 min)- and 1.12 (80 °C, 90 min)-fold for magnoflorine; 2.06–2.89-fold for baicalin; 3.01–3.84-fold for columbamine; 1.13–1.37-fold for epiberberine; 1.00–1.16-fold for jatrorrhizine; 11.71–19.53-fold for coptisine; 1.31–1.51-fold for wogonoside; 1.19–1.28-fold for palmatine; 27.31–44.13-fold for berberine; 2.04–2.96-old for wogonin; and 2.03–2.89-fold for oroxylin A, except for 0.90-fold for chrysin 6-C-arabinoside 8-C-glucoside (90 °C, 120 min) (Figures 3 and 4).

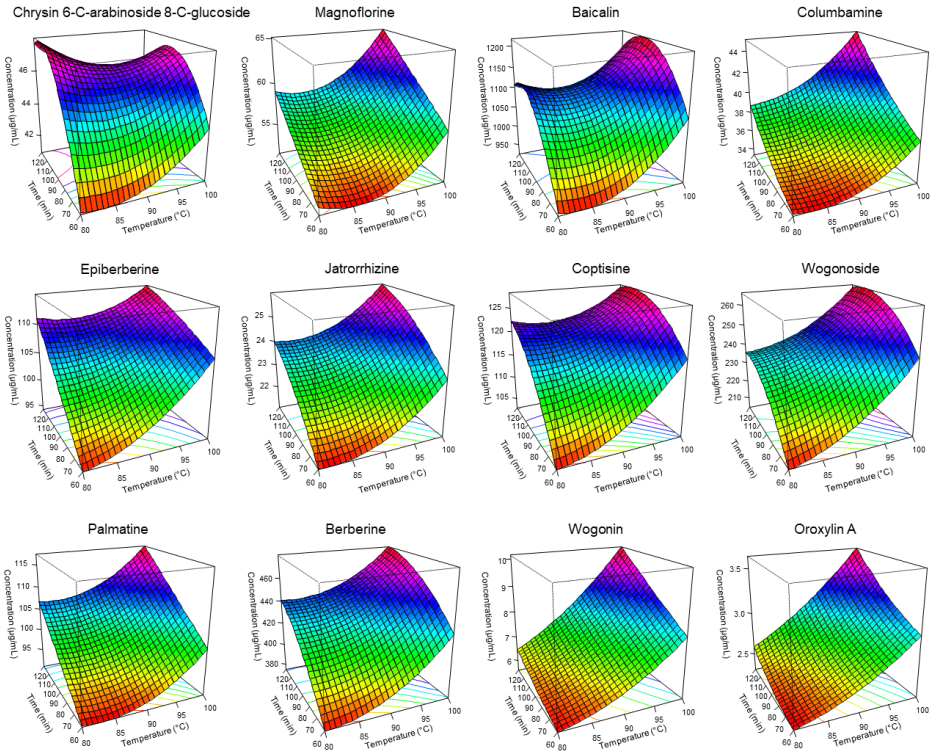

Figure 1. Response surface plots for the twelve marker compounds in a single extract of *S. baicalensis* or *C. chinensis* at various extraction temperatures and times. Color red is the lowest condition while color purple is the highest condition of parameters in 3D plot.

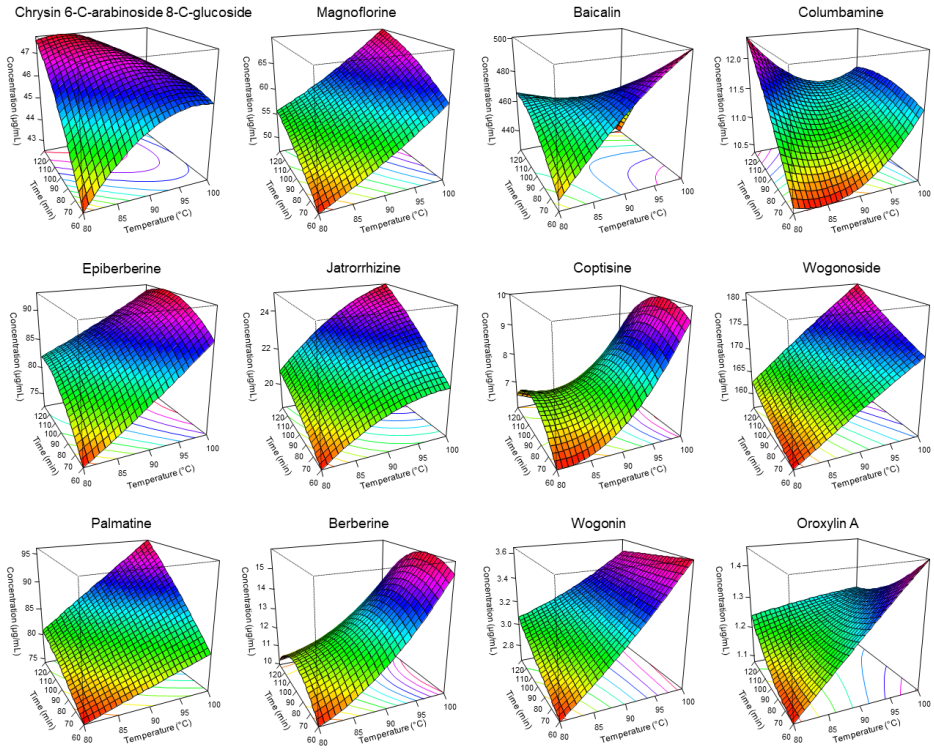

Figure 2. Response surface plots for the twelve marker compounds in *S. baicalensis* and *C. chinensis* single paired extract at various extraction temperatures and times. Color red is the lowest condition while color purple is the highest condition of parameters in 3D plot.

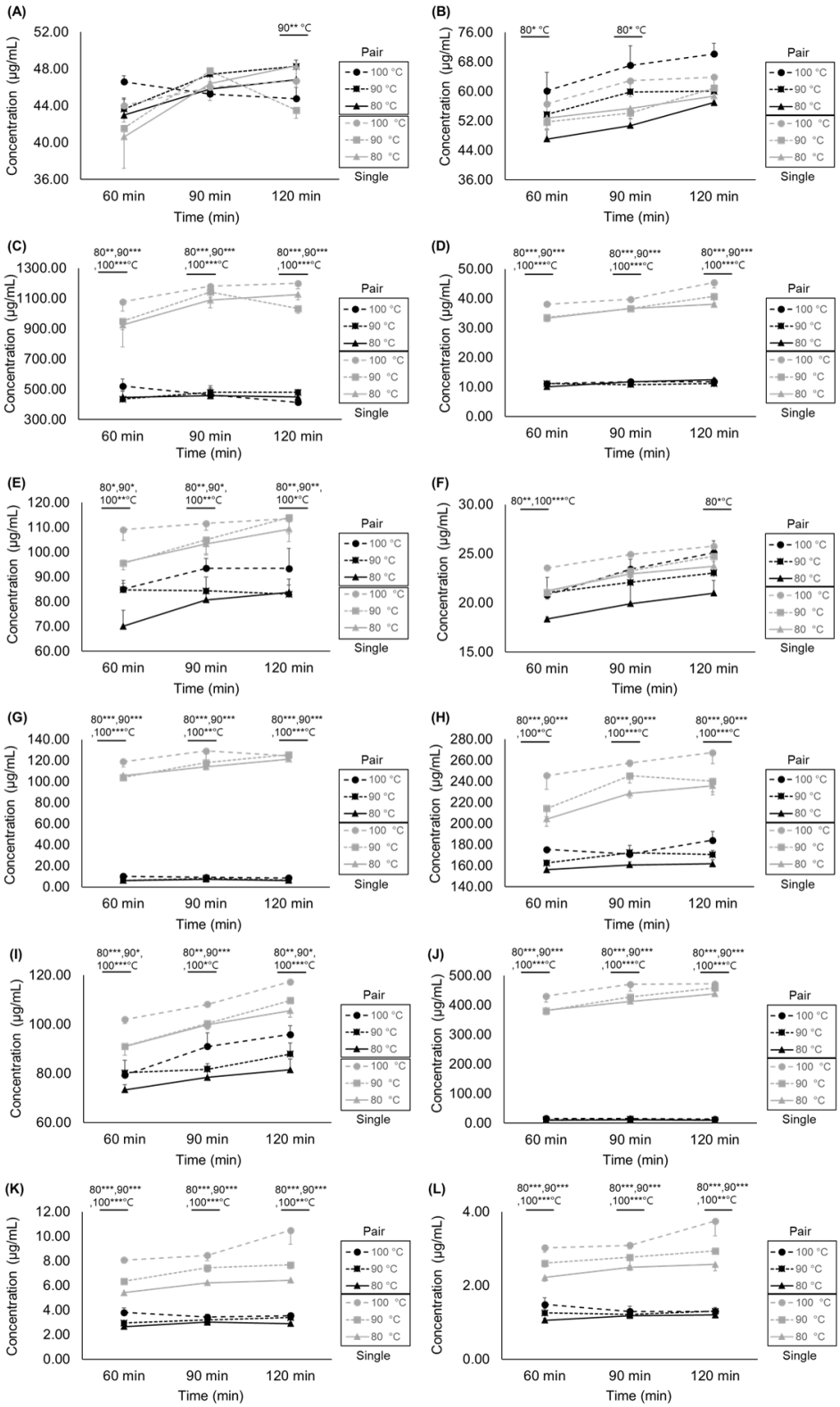

Figure 3. Changes in the contents of the marker compounds in the paired (black) or single extract (gray) of *S. baicalensis* and *C. chinensis* at various extraction times (60 min, 90 min, and 120 min). (**A**) Chrysin 6-C-arabinoside 8-C-glucoside; (**B**) magnoflorine; (**C**) baicalin; (**D**) columbamine; (**E**) epiberberine; (**F**) jatrorrhizine; (**G**) coptisine; (**H**) wogonoside; (**I**) palmatine; (**J**) berberine; (**K**) wogonin; (**L**) oroxylin A. Significance at *** $p < 0.001$, ** $p < 0.01$, or * $p < 0.05$.

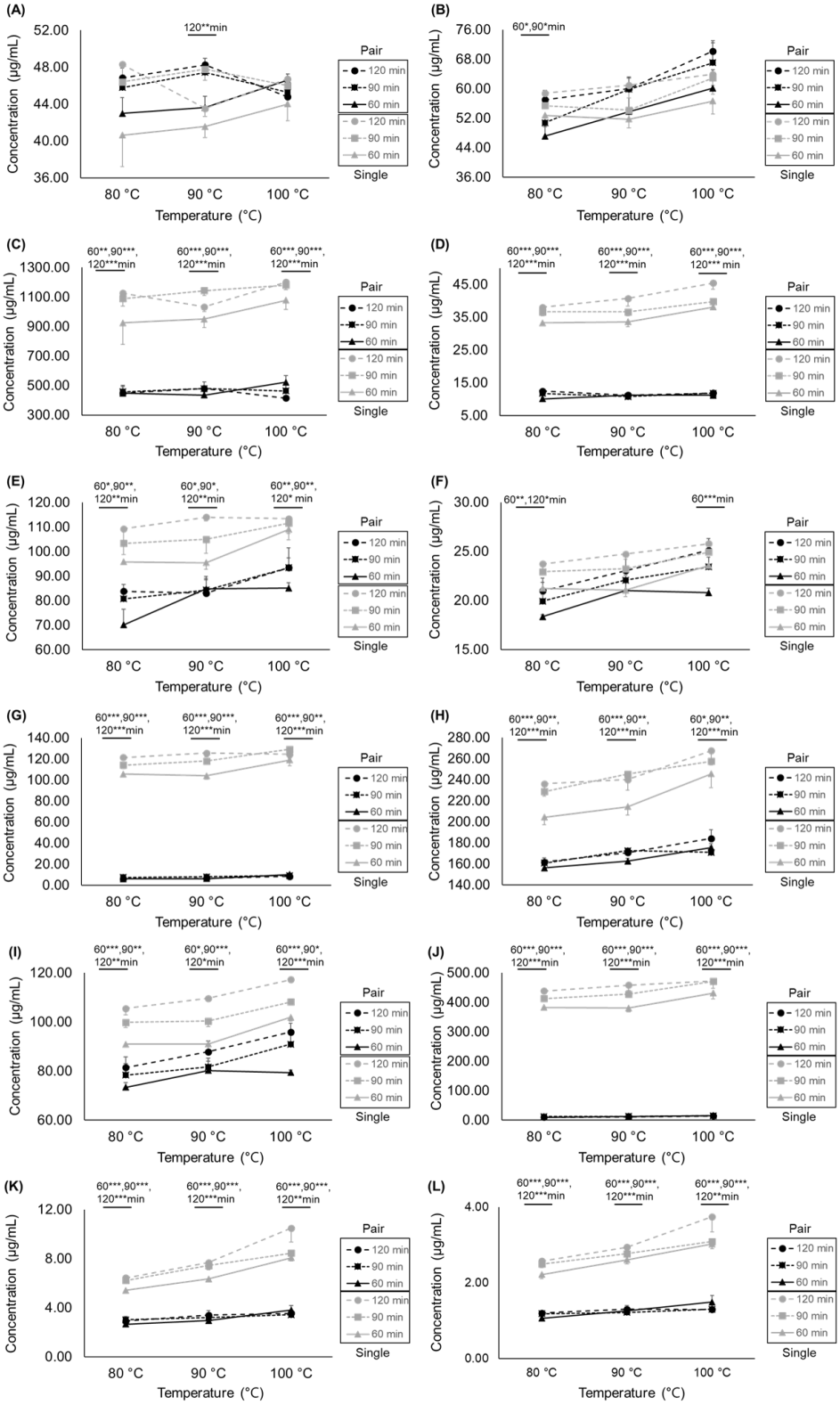

Figure 4. Changes in the contents of the marker compounds in the paired (black) or single extract (gray) *S. baicalensis* and *C. chinensis* at various extraction temperatures (80 °C, 90 °C, and 100 °C). (**A**) Chrysin 6-C-arabinoside 8-C-glucoside; (**B**) magnoflorine; (**C**) baicalin; (**D**) columbamine; (**E**) epiberberine; (**F**) jatrorrhizine; (**G**) coptisine; (**H**) wogonoside; (**I**) palmatine; (**J**) berberine; (**K**) wogonin; (**L**) oroxylin A. Significance at *** $p < 0.001$, ** $p < 0.01$, or * $p < 0.05$.

Particularly, significant reductions in the concentrations of four major compounds, baicalin, wogonoside, coptisine, and berberine, were evident when SB and CC were paired.

As previously reported in studies, extracting herbal combination alleviates the extraction efficiency of single herbal medicines [26]. This phenomenon can be explained by a decrease in the mass transfer of herbal compounds to extract due to a restriction of solvent access or a change in the solubility of constituents, both caused by added herbal medicine [27,28]. In contrast, by increasing the solubility of herbal compounds, herbal combinations may promote the extraction of constituents from the paired herb [29,30]. However, there may be another reason for the reduced concentrations of marker compounds in the paired extracts of SB and CC. As reported [3], when the herbal pair of SB and CC were decocted together in boiling water and then cooled, the components from the composing herbs gradually aggregated in the decoction as precipitation formed.

In addition, precipitation from an herbal formula containing SB and CC consisted of flavonoids from SB (including baicalin and wogonoside) and alkaloids from CC (including coptisine, berberine, and palmatine) [31–33]. Those flavonoids and alkaloids exist as complexes, such as baicalin-berberine or wogonoside-berberine, and these complexes were formed by combining an ionized carboxylic group of glucuronic acid (negative ion by hydrogen loss) in baicalin or wogonoside with ionized nitrogen of berberine (positive ion) [34]. According to these findings, the formation of the chemical complex can cause components in the extracted solution to precipitate naturally, resulting in a reduction in the number of components in the supernatant. This can explain why the presence of flavonoids and alkaloids in the paired extracts is significantly lower than in a single SB or CC extract. In addition, there is a possibility that higher extraction temperature (especially at 100 °C) and longer extraction time may make flavonoids and alkaloids, such as chrysin 6-C-arabinoside 8-C-glucoside, baicalin, coptisine, and berberine, more participate in forming the precipitation, as shown in Table 2.

The extraction efficiencies of 12 marker compounds were affected differently by extraction time and temperature when in the SB and CC single and paired extracts. Furthermore, the SB–CC pair is thought to have significantly reduced the extraction of the marker compounds from each herbal medicine by either co-interaction between the herbs or the formation of a precipitate. The precipitates consisting of flavonoids and alkaloids may be potent factors that accelerate the decrease in concentrations of the marker compounds in the extracted solution. Thus, the composition and formation mechanism of the precipitation will be investigated further in order to understand the combined effect of the SB–CC pair.

4. Concluding Remarks

The chemical influence of the SB–CC pair on the extraction yields of the 12 marker compounds at various extraction times (60, 90, and 120 min) and temperatures (80 °C, 90 °C, and 100 °C) was investigated using validated HPLC analysis with multiple regression analysis. In most paired and single extracts, the extraction efficiencies of the marker compounds improved in response to temperature and time increases. In contrast, the yields of baicalin, berberine, and coptisine were inversely proportional to the extraction time at 100 °C in the paired extracts. With the exception of chrysin 6-C-arabinoside 8-C-glucoside and magnoflorine, the concentrations of the marker compounds were significantly reduced by the SB–CC pair in most extraction conditions, compared to single extracts, possibly because of the formation of flavonoid alkaloid–complexed precipitation. The influence of extraction temperature and time on the formation of the flavonoid–alkaloid complex was not significant. This study identifies the chemical characteristics of the SB–CC pair, which is differently influenced by the extraction time and temperature from a single SB or CC extracts.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10020131/s1, Figure S1: Chemical structures of the twelve marker compounds found in a water extract of the roots of *Scutellaria baicalensis* and the rhizomes of *Coptis chinensis*.; Figure S2: Chromatograms of the twelve marker compounds (A), the

paired extract of *S. baicalensis* and *C. chinensis* (B), the single extract of *S. baicalensis* (C), and the single extract of *C. chinensis* (D) at a detection wavelength of 275 nm.; Table S1: Linear equations, correlation coefficients (r^2), LOD, LOQ, and the values of analytical method validation of the marker compounds.

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