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Analysis of Dibenzocyclooctadiene Lignans in Omija Wine and Cheong by Liquid Chromatography-Tandem Mass Spectrometry

Hyung-Ju Seo,[1] Seung-Bae Ji,[1] Sin-Eun Kim,[1] Gyung-Min Lee,[1] Seong-Hun Moon,[2] Dae-Sik Jang,[3]* and Kwang-Hyeon Liu[1]*

[1]BK21 Plus KNU Multi-Omics based Creative Drug Research Team, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University; Daegu 41566, Korea
[2]OmyNara 609, Saejaero, Mungyeong 36919, Kyungsangbukdo, Korea
[3]Department of Life and Nanopharmaceutical Sciences, KyungHee University, Seoul 02447, Korea

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Abstract : *Schisandra chinensis* is a traditional herbal medicine that is widely spread in Korea, Japan, and China. The fruits of *S. chinensis* Bailon, known as omija in Korea, have traditionally been used for the treatment of coughs, fatigues, and insomnia. Up to now, there have been several reports for the identification of major lignan compounds and their quantitation in *S. chinensis* extracts. To the best of our knowledge, however, there is no report on the analysis of lignans in omija wine and omija cheong (sugared omija or omija sugar syrup). In the present study, seven dibenzocyclooctadiene lignans (gomisin A, gomisin B, gomisin C, gomisin N, schisandrin, deoxyschisandrin, and wuweizisu C) in omija wine and omija cheong were analyzed and quantitated using liquid chromatography-tandem mass spectrometry. Among seven lignans, pharmacologically active gomisin A, schisandrin, and deoxyschisandrin, which are major components in fruits of *S. chinensis*, were the most abundant lignans in omija wine and cheong. The content of lignan in omija wine was in the order: schisandrin > gomisin A > deoxyschisandrin > gomisin N > gomisin B > gomisin C > wuweizisu C. The concentration of deoxyschisandrin and gomisin N in omija wine was approximately 2.0- and 6.0-fold higher than for omija cheong. Additionally, this study provided a systematic identification of lignans in omija wine and cheong and indicated that the omija wine and cheong might be of value for their dietary application.

Key words : Lignan, Liquid chromatography-tandem mass spectrometry, Omija wine, *Schisandra Chinensis* Baillon

Introduction

Schisandra chinensis Bailon, a member of Schisandraceae family, is a climbing plant widely distributed in eastern Asia including Korea, Japan and China.[1]The fruits of *S. chinensis* Bailon, known as omija in Korea, have traditionally been used for the treatment of coughs, fatigues, spontaneous sweating, dysentery, and insomnia,[2] and used as a food additive for beverages, sugared omija, and wine.[3,4] The literal meaning of omija is "berries with five distinctive flavor characteristics: astringency, bitterness, saltiness, sourness, and sweetness,[5] and the aqueous extract of this fruit has a pinkish-red color. Omija extract has been reported to have hepatocellular protective,[6] liver function restorative,[7] whitening,[8] anti-bacterial,[9] anti-inflammatory,[10] anti-oxidant,[11] and anti-cancer[12] effects in many previous studies. Particularly, it has been suggested that gomisin N has a specific anti-proliferative effect and pro-apoptotic effect on liver cancer cells.[13] Gomisin A and gomisin C, dibenzocyclooctadiene lignans with aromatic methylenedioxy groups and hydroxyl groups, are known to help prevent neurodegenerative disease and dementia like Alzheimer's diseases.[14] In addition, schisandrin exhibits hypoglycemic action, anti-ulcer action, and potent anti-viral activities in chronic hepatitis.[15] The sum of the contents of gomisin A, gomisin N, and schisandrin is used as an index component.[16]

Up to now, there have been several reports for the identification of major lignan compounds and their quantitation in *S. chinensis* extracts.[2,10,17-21] To the best of our knowledge, however, there is no report on the analysis of lignans in omija wine and omija cheong (sugared omija or omija sugar syrup). In the present study, seven dibenzocyclooctadiene lignans (gomisin A, gomisin B, gomisin C, gomisin N, schisandrin, deoxyschisandrin, and wuweizisu C, Figure 1) in omija wine and omija cheong

*Reprint requests to Kwang-Hyeon Liu, Dae-Sik Jang
E-mail: dstlkh@knu.ac.kr, dsjang@khu.ac.kr

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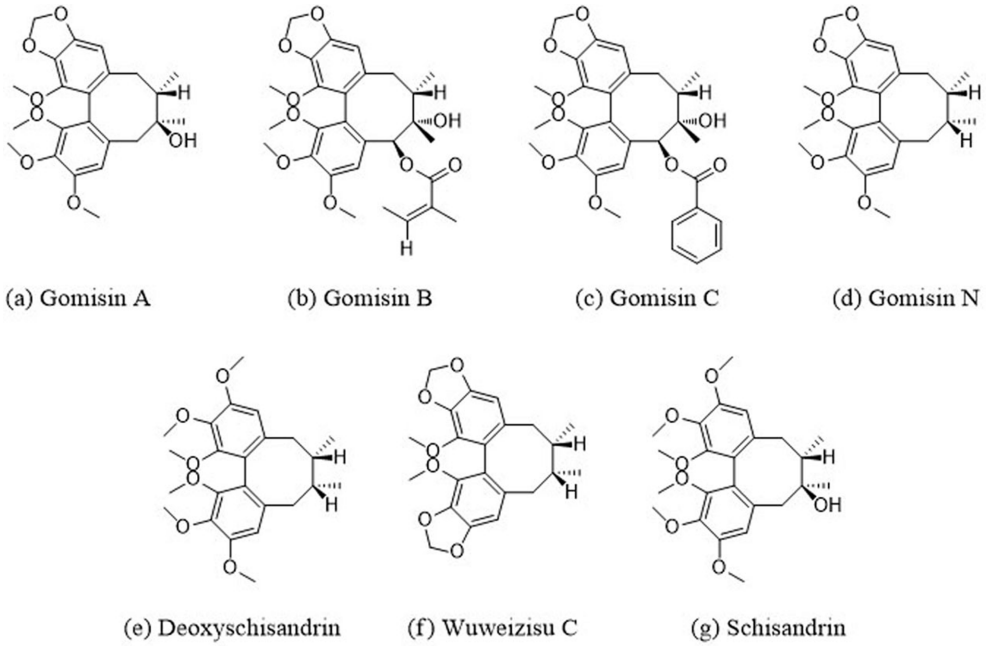

Figure 1. Chemical structures of the dibenzocyclooctadiene lignans: (a) gomisin A; (b) gomisin B; (c) gomisin C; (d) gomisin N; (e) deoxyschisandrin; (f) wuweizisu C; and (g) schisandrin.

were analyzed and quantitated using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Experimental

Materials

Omija wine (Omy Rosé®) and omija cheong (Omy Rosé N®) were obtained from OmyNara (Mungyeong, Korea). Gomisin A, gomisin N, schisandrin, deoxyschisandrin, and wuweizisu C were isolated from fruits of *Schisandra chinensis* Bailon, and their chemical structures were identified by analyzing their NMR data, which were in good agreement with those published in our previous report.[2] In this paper, we have adopted the nomenclature of lignans from the recent review article.[22] Gomisin B and gomisin C were purchased from Toronto Research Chemical (Toronto, Canada) and Sigma-Aldrich (St. Louis, MO, USA), respectively. All the other solvents were LC-MS grade (Fisher Scientific Co., Pittsburgh, PA, USA).

Preparation of sample extract

Dichloromethane extracts were prepared from omija wine and cheong. The samples (30 mL, three replicates) were extracted with 50 mL of dichloromethane. The procedure was carried out three times and the organic layer was concentrated using a rotary evaporator (Eyela, Japan). The concentrate was reconstituted at 1 mL of methanol. All samples were then filtered using 0.2 μm membrane filters before analysis by LC-MS/MS.

Linearity and calibration curve

Seven lignan standards (gomisin A, gomisin B, gomisin C, gomisin N, schisandrin, deoxyschisandrin, and wuweizisu C, Figure 1) were dissolved in methanol and were diluted stepwise for calibration solutions. The analysis was performed under established LC-MS/MS conditions and the calibration curve was prepared in the form of $y = ax + b$ (y: peak area, x: lignan concentration) (Table 1).

LC-MS/MS analysis

Omija wine and cheong extracts were analyzed using a LC-MS/MS (LCMS 8040, Shimadzu, Kyoto, Japan) equipped with an electrospray ionization interface. The samples were separated on a Kinetex C18 column (100 × 2.10 mm, 2.6 μm, 100 Å; Phenomenex, Torrance, CA, USA). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), and was set as 55% B (0–4 min), 55% → 90% B (4–6 min), 90% B (6–8 min), 90% → 55% B (8–8.1 min) and 55% B (8.1–10 min). The total run time was 10 min, and the flow rate was 0.2 mL/min. Electrospray ionization was performed in positiveion mode at 4000 V. The optimum operating conditions were determined as follows: capillary temperature, 350°C; vaporizer temperature, 300°C; collision gas (argon) pressure, 1.5 mTorr. Quantitation was conducted in selected reaction monitoring (SRM) modes with the precursor to product ion transition (Table 2).

Table 1. Optimized selected reaction monitoring parameters for seven lignans used in all the assay.

Lignan	Molecular formula	Parent ion (*m/z*)	Fragment ion (*m/z*)	Polarity	Collision Energy (eV)
Gomisin A	$C_{23}H_{28}O_7$	417.3 $[M+H]^+$	399.3	*ESI^+	20
Gomisin B	$C_{28}H_{34}O_9$	537.3 $[M+Na]^+$	415.3	ESI^+	25
Gomisin C	$C_{30}H_{32}O_9$	554.3 $[M+NH_4]^+$	415.3	ESI^+	20
Gomisin N	$C_{23}H_{28}O_6$	401.3 $[M+H]^+$	300.3	ESI^+	25
Deoxyschisandrin	$C_{24}H_{32}O_6$	417.3 $[M+H]^+$	316.3	ESI^+	25
Wuweizisu C	$C_{22}H_{24}O_6$	385.3 $[M+H]^+$	285.3	ESI^+	25
Schisandrin	$C_{24}H_{32}O_7$	433.3 $[M+H]^+$	415.3	ESI^+	25

* ESI: Electrospray ionization

Table 2. Calibration data of seven lignans and contents of seven lignans in omija cheong and omija wine.

Lignan	Linear range (ng/ml)	Regression equation $y = ax + b$	r^2	LOQ (ng/ml)	Contents (ng/ml of product) (Mean ± SD, $n = 3$)	
					Omija cheong	Omija wine
Gomisin A	416-41,600	$y = 51{,}889x + 4{,}841$	0.9943	30	457.87 ± 123.40	215.77 ± 18.00
Gomisin B	51-2,570	$y = 100{,}026x + 1{,}330$	0.9920	15	34.27 ± 10.63	24.33 ± 0.70
Gomisin C	5-540	$y = 1{,}427{,}640x + 3{,}795$	0.9945	5	1.97 ± 0.70	4.77 ± 0.37
Gomisin N	40-4,000	$y = 475{,}795x + 9{,}560$	0.9991	5	7.73 ± 4.00	49.33± 5.20
Deoxyschisandrin	416-20,800	$y = 962{,}658x + 272{,}168$	0.9961	5	59.10± 22.03	113.00 ± 8.47
Wuweizisu C	5-380	$y = 194{,}267x + 411$	0.9939	5	11.52± 3.84	4.60 ± 1.53
Schisandrin	2,160-86,400	$y = 85{,}139x + 76{,}300$	0.9925	40	3,123.37 ± 338.53	1,261.73 ± 44.50

Results and Discussion

In this study, we analyzed seven dibenzocyclooctadiene lignans (gomisin A, gomisin B, gomisin C, gomisin N, schisandrin, deoxyschisandrin, and wuweizisu C) which were reported as bioactive components in omija,[17] in omija wine and cheong sample using LC-MS/MS. Precursor ions for gomisin A, gomisin B, gomisin C, gomisin N, schisandrin, deoxyschisandrin, and wuweizisu C, and their corresponding product ions were determined from spectra obtained during the infusion of standard solutions into a mass spectrometer using an electrospray ionization source, which operated in positive ionization mode. Gomisin A, gomisin N, schisandrin, deoxyschisandrin, and wuweizisu C mainly produced protonated molecular ions ($[M+H]^+$) at *m/z* 417.3, 401.3, 433.3, 417.3, and 385.3, respectively. Gomisin B and gomisin C were observed at *m/z* 537.3 and 554.3 as sodium adduct ($[M+Na]^+$) and ammonium adduct ($[M+NH_4]^+$), respectively. Their product ions were scanned in Q3 after collision with nitrogen in Q2 at *m/z* 399.3 for gomisin A, at *m/z* 415.3 for gomisin B, gomisin C, and schisandrin, at *m/z* 300.3 for gomisin N, *m/z* 316.3 for deoxyschisandrin, and *m/z* 285.3 for wuweizisu C (Figure 2). These are the most sensitive product ions for lignan quantification (Figure 2). The fragment ion at *m/z* 300.1., 316.1, and 284.9 for gomisin N, deoxyschisandrin, and wuweizisu C, respectively, corresponding to the loss of $C_6H_{13}O$, were formed by the combined losses of C_5H_{10} at the eight-membered dibenzene ring and one OCH_3 at benzene ring.[23] The precursor (Q1) and product ions (Q3) selected for each lignan are given in Table 1, together with the optimal collision energy.

Figure 3 shows the representative SRM chromatograms for lignan standards (left column), omija cheong extracts (middle column), and omija wine extracts (right column). As indicated in Figure 3, seven lignans were eluted in less than 8 min and separated in their individual SRM channels. Under the described chromatographic conditions, the retention times of gomisin A, gomisin B, gomisin C, gomisin N, schisandrin, deoxyschisandrin, and wuweizisu C were 3.2, 5.9, 5.8, 7.7, 2.6, 7.3, and 7.8 min, respectively. The linearity of the calibration curve was assessed by plotting the peak area (y) versus the concentration (x) of the calibration standards using weighted ($1/x^2$) least square linear regression.[24] The mean correlation coefficient (r^2) of the respective weighted calibration curves were over 0.992 (Table 2). The limit of quantification (LOQ) were determined as follows: LOQ = 10 × signal to noise ratio.[25] The LOQ for gomisin A, gomisin B, gomisin C, gomisin N, schisandrin, deoxyschisandrin, and wuweizisu C were 30, 15, 5, 5, 40, 5, and 5 ng/ml, respectively (Table 2).

Szopa et al.[10] reported the contents of schisandrin, deoxyschisandrin, gomisin A, gomisin B, gomisin C, gomisin D, gomisin G, gomisin J, gomisin N, gomisin K_3,

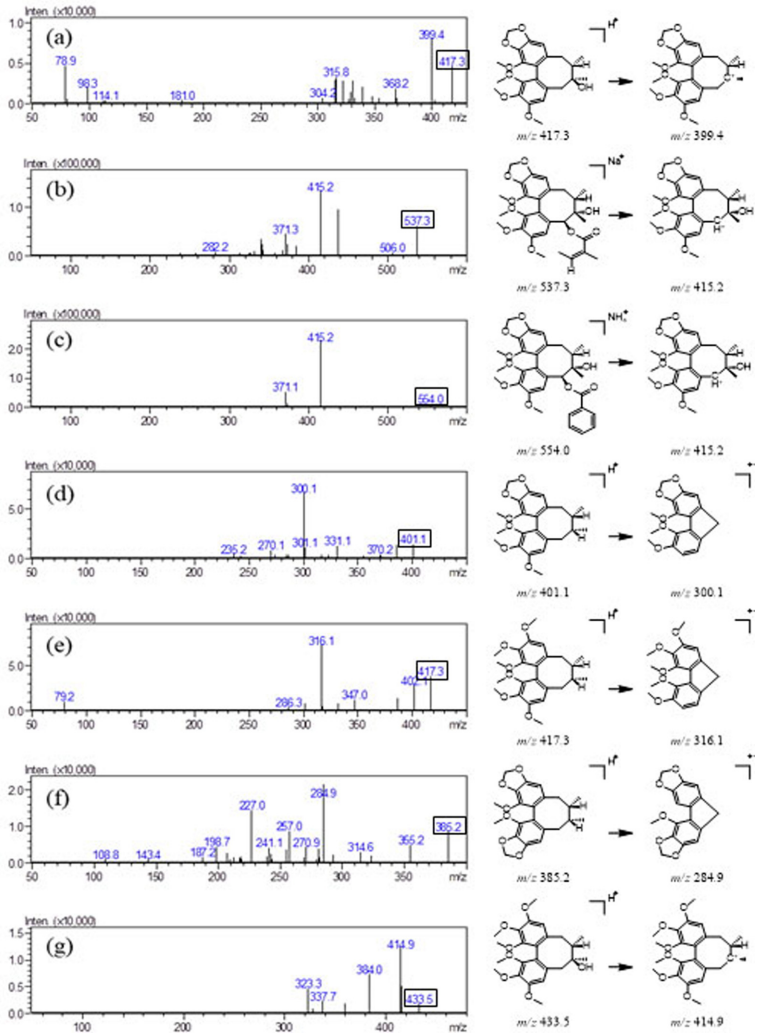

Figure 2. Product ion scan mass spectra of seven lignans (a) gomisin A, (b) gomisin B, (c) gomisin C, (d) gomisin N, (e) deoxyschisandrin, (f) wuweizisu C, and (g) schisandrin obtained using electrospray ionization mode.

angeloylgomisin H, benzoylgomisin O, and wuweizisu C in fruit, leaf, and shoot extracts of *S. chinensis*. Liu et al.[18] also reported that the contents of schisandrin, deoxyschisandrin, g-schisandrin, gomisin A, gomisin C, gomisin G, gomisin K_3, angeloylgomisin H, and wuweizisu C in fruits of *S. chinensis*. Schisandrin, deoxyschisandrin, g-schisandrin, and gomisin A were identified as major components in fruits of *S. chinensis*.[19,21] These lignans exhibited anti-inflammatory, antioxidant, antihepatotoxic, antitumor, and antiviral activity as well as effects on cognitive function, physical performance and central nervous system.[1,14,15,18,26-28]. Despite lignans' biological importance, little is known about the lignan contents in omija wine and omija cheong (sugared omija or omija sugar syrup). Like pharmacologically active resveratrol contents in grape wine[29-31] there is need for the lignan composition of omija wine and omija cheong because of the increase of consumers' interest that functional foods have acquired in recent years.

In the present study, we analyzed seven major lignans (gomisin A, gomisin B, gomisin C, gomisin N, schisandrin, deoxyschisandrin, and wuweizisu C) in omija wine and omija cheong using LC-MS/MS. Information on the levels of seven lignans in omija wine and omija cheong is presented in Table 2. Typical SRM chromatograms obtained in the analysis of seven lignans in omija wine and omija cheong are illustrated in Figure 3. Omija wine and cheong contained much higher levels of gomisin A and schisandrin, which are index components of omija specified in the Korean Pharmacopoeia[16] than other lignans. Gomisin A has been known to reduce metastatic melanoma and colorectal cancer through its anti-proliferative and anti-metastatic activities.[32,33] Schisandrin is expected to be a potential drug for improving Parkinson's disease.[34] The content of lignan in omija wine

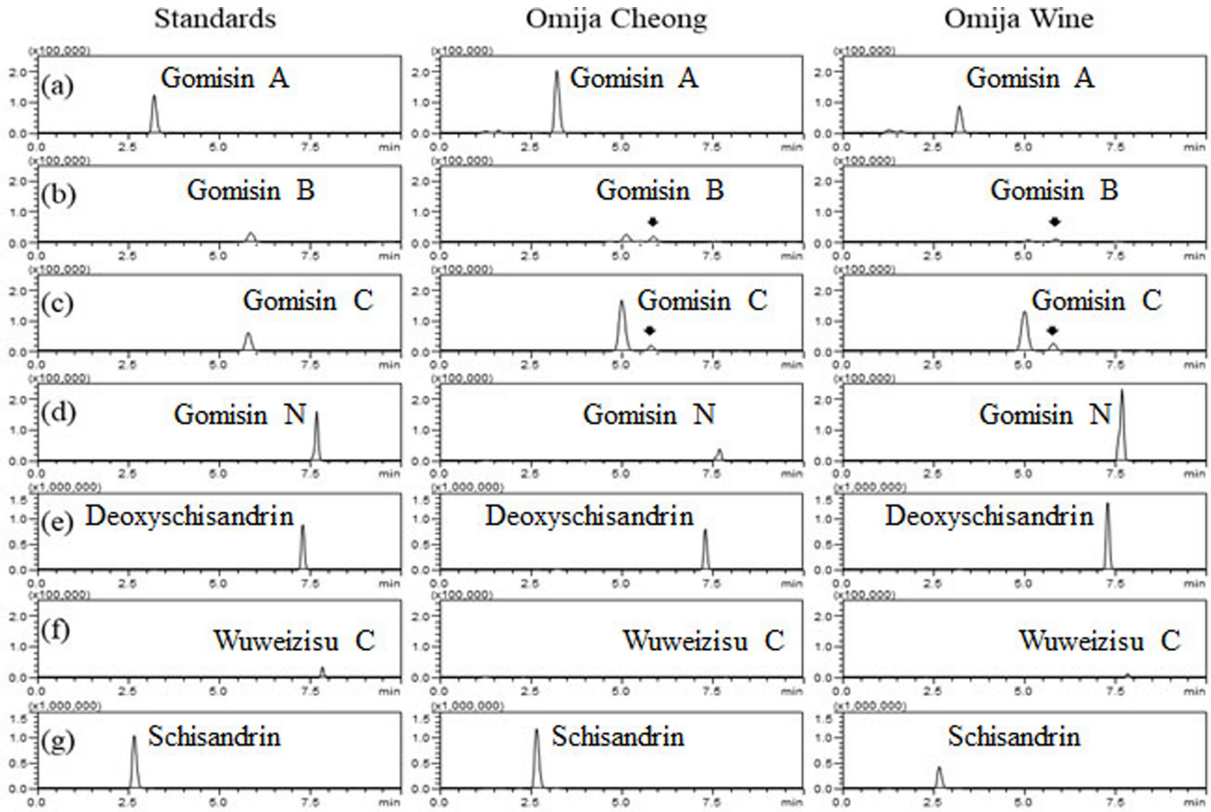

Figure 3. Representative selected reaction monitoring chromatograms of seven lignan standards (left column), omija cheong extracts (middle column), and omija wine extracts (right column). Gomisin A (a, 3.2 min), gomisin B (b, 5.9 min), gomisin C (c, 5.8 min), gomisin N (d, 7.7 min), deoxyschisandrin (e, 7.3 min), wuweizisu C (f, 7.8 min), and schisandrin (g, 2.6 min) was monitored in positive electrospray ionization mode.

was in the order: schisandrin > gomisin A > deoxyschisandrin > gomisin N > gomisin B > gomisin C > wuweizisu C, whereas that in omija cheong was in the order: schisandrin > gomisin A > deoxyschisandrin > gomisin B > wuweizisu C > gomisin N > gomisin C (Table 2). The concentration of deoxyschisandrin and gomisin N in omija wine was approximately 2.0- and 6.0-fold higher than for omija cheong, while that of schisandrin and gomisin A in omija cheong was approximately 2.5- and 2.0-fold higher than for omija wine (Table 2).

Conclusions

The present study is the first comparative and quantitative analyses of lignans in omija wine and cheong. The study identified seven dibenzocyclooctadiene lignans (gomisin A, gomisin B, gomisin C, gomisin N, schisandrin, deoxyschisandrin, and wuweizisu C) in omija wine and cheong. Pharmacologically active gomisin A, schisandrin, and deoxyschisandrin, which are major compounds in fruits of *Schisandra chinensis,* were the most abundant lignans in both samples. In the future, the composition of lignans in the omija wine and cheong may be of value for their dietary and nutritional application.

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