Lee *et al. Applied Biological Chemistry* (2022) 65:77
https://doi.org/10.1186/s13765-022-00746-2

KSABC
The Korean Society for Applied Biological Chemistry

ARTICLE

Open Access

The growth characteristics and lignans contents of *Schisandra chinensis* fruits from different cultivation regions

Dong Hwan Lee[1], Youngki Park[2], Jun Hyuk Jang[1], Yonghwan Son[1], Ji Ah Kim[1], Sun-Young Lee[1] and Hyun-Jun Kim[1*]

Abstract

We developed and validated an optimized method for quantifying lignans using ultra-performance liquid chromatography (UPLC) and performs correlation analysis of growth characteristics and contents of lignans. The methods for determining lignans were validated by measuring the linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision using UPLC. All calibration curves showed good linearity ($r^2 \geq 0.9998$) within the tested ranges. The limit of detection (LOD) and limit of quantification (LOQ) were 0.003–0.02 μg/mL and 0.01–0.07 μg/mL, respectively. The precision of analysis was less than 3%. The recoveries of quantified compounds ranged from 98.4 to 101.68%. Growth characteristics of fruits were negatively correlated with content of total marker compounds. The results of this study can be used to quality-control of *S. chinensis* fruits used as medicinal raw materials.

Keywords: Growth characteristic, Lignan, Method validation, *Schisandra chinensis*, UPLC-UV

Introduction

Schisandra chinensis, belongs to the *Schisandraceae* family, is mainly found in Korea and its neighboring regions, i.e. Japan, Northern China, and Russian Far East [1]. It has been used in traditional herbal medicine for centuries in Asia [2]. In Asia, *Schisandraceae* has been used in the treatment of chronic coughs and asthma, enuresis, diabetes symptoms, diarrhea, etc. [3–5]. Also, various physiological functions such as immunomodulatory and antibacterial activity, blood pressure lowering and alcohol detoxification activity, liver-protecting, antitumor, anti-HIV, antioxidant activity, antiaging, and regulation of the central nervous system have been reported as the efficacy of *S. chinensis* [6–12]. Currently, studies are being conducted on the main components of *Schisandra*, mainly composed of lignans such as schisandrin, gomisin, deoxyschisandrin, schisandrol, etc. [13]. Lignan is a class of secondary metabolites produced by oxidative dimerization of two phenylpropanoid units. They are widely distributed in the plant kingdom and have been found in species belonging to more than seventy families [14]. Studies on the activity of these components have also been conducted, lignans in *S. chinensis* have a common dibenzocyclooctadiene skeleton structure, and this group is evaluated to have antioxidant and antiproliferative effects on human cancer cells [15, 16].

The studies on growth characteristics have been reported and have also been reported study on lignan contents of *S. chinensis* fruit [17, 18]. But, current cultivation sites often don't suit optimum conditions needed for the increased production of active compounds. The active compounds of medicinal plants vary in composition and content depending on the growth period, soil physicochemical properties and the environment, such as temperature, precipitation. In general, the growth environment for plants affects the growth characteristics of the fruits, and the content of the active compounds varies

*Correspondence: mind4938@korea.kr

[1] Forest Medicinal Resources Research Center, National Institute of Forest Science, Yeongju 36040, South Korea
Full list of author information is available at the end of the article

depending on the growth characteristics [19, 20]. Therefore, research on the environmental factors affecting the content of active compounds is needed to improve the quality of locally produced *S. chinensis* as a functional raw material. In this study, we aimed to investigate the correlation of lignans and growth characteristics of *S. chinensis* fruits that can be used as raw materials, prior to the study with the environment. Afterwards, these results will use as basic data for the standard cultural practices of *S. chinensis*.

Table 1 Geographic information about the cultivation regions where fruits of *Schisandra chinensis* were collected in South Korea

Cultivation regions	Name of regions	Altitude (m)	N (latitude)	E (longitude)
1	Taebaek-si	470	37° 15′ 41.0″	128° 59′ 42.0″
2	Pyeongchang-gun	566	37° 24′ 44.0″	128° 28′ 50.0″
3	Hongcheon-gun	500	37° 49′ 07.0″	128° 27′ 16.0″
4	Yongin-si	154	37° 18′ 58.0″	127° 12′ 33.0″
5	Sancheong-gun	112	35° 24′ 38.0″	127° 47′ 23.0″
6	Hamyang-gun	146	35° 32′ 23.0″	127° 36′ 49.0″
7	Gyeongju-si	170	35° 45′ 29.0″	128° 58′ 53.0″
8	Mungyeong-si	80	36° 41′ 09.0″	128° 03′ 36.0″
9	Mungyeong-si	99	36° 46′ 00.0″	128° 21′ 51.0″
10	Mungyeong-si	506	36° 37′ 46.0″	128° 03′ 30.0″
11	Uiseong-gun	756	36° 29′ 47.0″	128° 27′ 48.0″
12	Muju-gun	460	35° 55′ 48.3″	127° 42′ 22.0″
13	Muju-gun	284	35° 52′ 02.6″	127° 38′ 36.7″
14	Muju-gun	560	35° 55′ 48.3″	127° 42′ 22.0″
15	Gongju-si	503	36° 38′ 12.0″	126° 58′ 58.0″
16	Nonsan-si	394	36° 06′ 46.0″	127° 14′ 00.0″
17	Seosan-si	226	36° 50′ 07.0″	126° 24′ 35.0″
18	Jecheon-si	130	36° 53′ 52.0″	128° 09′ 15.0″
19	Cheongju-si	93	36° 36′ 13.0″	127° 37′ 59.0″

Material and methods

Plant materials and chemicals

In this study, a total of 95 specimens of *S. chinensis* fruits were collected from 19 plantations in 15 regions across the South Korea in September, 2020 (Table 1). The samples have been identified by the taxonomic identification for the samples were performed and stored at 2 °C. In the Korean Pharmacopoeia [21], schisandrin, gomisin A and gomisin N were selected as marker compounds of *S. chinensis* fruits (Fig. 1). Schisandrin and gomisin A were purchased from Sigma-Aldrich (MO, USA). Gomisin N was purchased from ChemFaces (Hubei, China). HPLC-grade methanol, ethanol, acetonitrile and distilled water were purchased from J.T. Baker (PA, USA) and used without purification.

Growth characteristics

FruitS of *S. chinensis* showed moisture content of 81.18 ± 2.38%. Quantitative characteristics of *S. chinensis* such as number of fruits per fruit bunch, length of fruit bunch, width of fruit bunch, fresh weight of fruit bunch, length of fruit, width of fruit, fresh weight of fruit, fresh weight of 30 fruits, and sugar contents of fruit were measured using digital calipers (500-182-30, Mitutoyo Co. Japan) and electronic scale (HS3200S, HANSUNG instrument Co. Korea).

Sample and standard preparation

The collected samples were washed with distilled water and then lyophilized. After measuring the dry weight of the sample, the powder was pulverized with a grinder (KSP-35, Korea Medi Co. Ltd., Korea), and then passed through an 80 mesh standard sieve and stored at -18℃ and used as an analysis sample. As for the sample

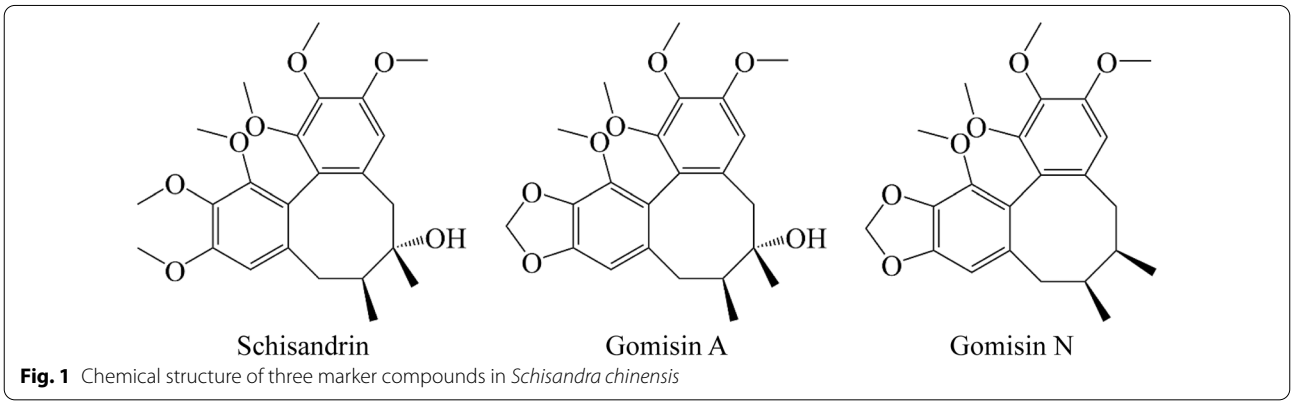

Fig. 1 Chemical structure of three marker compounds in *Schisandra chinensis*

Table 2 Linear regression, LOD, LOQ of three marker compounds

Compound	Regression equation	Correlation coefficient (r^2)	Range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
Schisandrin	Y = 7056.3X + 25002	0.9998	6.25–800	0.003	0.01
Gomisin A	Y = 2679.4X + 5292.2	0.9998	6.25–800	0.02	0.07
Gomisin N	Y = 5901.4X + 3544.5	0.9999	6.25–800	0.01	0.04

Table 3 Intra-, Inter-day precision of three marker compounds

Compound	Concentration (μg/mL)	Intra-day[a] ($n = 3$)		Inter-day[b] ($n = 3$)	
		Concentration found (μg/mL)	RSD (%)	Concentration found (μg/mL)	RSD (%)
Schisandrin	25	22.8	0.02	22.7	2.50
	100	103.9	0.52	101.8	1.70
	400	414.0	0.14	410.5	0.62
Gomisin A	25	25.8	0.05	26.2	1.54
	100	98.7	0.08	101.3	2.34
	400	399.8	0.09	409.5	1.98
Gomisin N	25	24.3	0.02	23.4	0.68
	100	102.8	0.02	96.8	0.23
	400	403.9	0.07	397.1	0.85

[a] Sample analyzed three times on 1 day, n = 3

[b] Sample analyzed each day on three time consecutive days, n = 3

extraction method, the quantitative method specified in the Korean Pharmacopoeia was little bit modified [21]. A sample of the powder (500 mg) was extracted with 10 mL of 100% methanol in an ultrasonic bath (JAC-5020, KODO, Korea. An ultrasonic output power and frequency of the ultrasonic bath were 350 W and 40 kHz, respectively. The sonication temperature and time were 30 ℃ and 60 min. After extraction, the sample was centrifuged (Labogene, BMS, Korea) at 3000 rpm for 10 min, and the supernatant was separated. The supernatant was filtered by a 0.45 μm membrane filter (Whatman co., Maidstone, UK). Standards (schisandrin, gomisin A and gomisin N) stock solutions for UPLC were prepared by diluting the stock solutions in methanol to obtain concentration ranges of 6.25–800 μg/mL for three marker compounds.

Table 4 Recoveries of three marker compounds

Compound	Concentration (μg/mL)	Recovery[a] (%) ($n = 3$)	RSD (%)
Schisandrin	6.25	98.40	2.81
	25	100.18	1.37
	100	100.81	0.38
Gomisin A	6.25	101.14	1.41
	25	100.48	1.40
	100	100.42	1.47
Gomisin N	6.25	99.91	0.38
	25	101.68	1.19
	100	101.27	1.43

[a] Recovery (%) = [(amount from sample spiked standard–amount from sample)/amount from spiked standard] × 10

UPLC conditions

Analysis data were obtained using a Waters alliance UPLC® (Waters co., MA, USA) with a UV detector. The analytical conditions for recording chromatograms of the three compounds were as follows: Qualitative and quantitative analyses were carried out using an ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm, 130Å, Waters co., MA, USA) with a column oven at 30 ℃. The mobile phase was a binary eluent of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with gradient conditions as follows: Initial——3 min, 45% B; 3–5 min, 52% B; 5–6 min, 53% B; 6–8 min, 58% B; 8–10 min, 64% B; 10–15 min, 64% B; 15–15.1 min, 76% B; 15.1–17 min, 100% B; injection volume of 1 μL, flow rate of 0.3 mL/min and detection wavelength of 254 nm. Each sample was analyzed in triplicate and expressed as a mean value.

Table 5 Growth characteristics of *Schisandra chinensis* fruit in 19 different cultivation regions

Cultivation regions	Number of fruits per fruit bunch	Length of fruit bunch	Width of fruit bunch	Fresh weight of fruit bunch	Length of fruit	Width of fruit	Fresh weight of fruit	Fresh weight of 30 fruits	Sugar contents of fruit
		(mm)	(mm)	(g)	(mm)	(mm)	(g)	(g)	(Brix°)
1	30.87 ± 3.25^{a}	63.83 ± 2.07^{abc}	23.31 ± 1.63^{abc}	17.92 ± 3.08^{ab}	11.17 ± 0.61^{ab}	9.51 ± 0.36^{abc}	0.68 ± 0.08^{cde}	22.80 ± 1.12^{abc}	8.62 ± 1.68^{abcde}
2	26.27 ± 2.63^{ab}	77.61 ± 12.08^{a}	23.99 ± 2.59^{ab}	20.09 ± 2.88^{a}	12.31 ± 0.39^{a}	10.58 ± 0.33^{a}	0.90 ± 0.03^{ab}	27.35 ± 2.16^{ab}	10.63 ± 0.93^{a}
3	27.40 ± 2.18^{ab}	72.60 ± 2.80^{ab}	22.23 ± 5.23^{abcd}	17.50 ± 5.38^{ab}	10.62 ± 1.52^{bc}	9.22 ± 1.39^{abc}	0.74 ± 0.23^{bcd}	22.38 ± 7.20^{abc}	8.99 ± 1.44^{abcd}
4	29.33 ± 4.10^{ab}	68.16 ± 5.19^{abc}	20.94 ± 1.99^{abcd}	15.20 ± 2.64^{ab}	10.25 ± 0.86^{bc}	9.21 ± 0.54^{bc}	0.63 ± 0.05^{de}	19.63 ± 2.62^{c}	9.07 ± 1.43^{abcd}
5	23.53 ± 3.19^{b}	56.79 ± 9.81^{c}	19.70 ± 2.13^{d}	11.41 ± 3.43^{b}	9.27 ± 0.68^{c}	8.67 ± 0.67^{c}	0.54 ± 0.08^{e}	18.00 ± 1.54^{c}	5.33 ± 1.16^{g}
6	28.53 ± 4.09^{ab}	72.37 ± 4.89^{ab}	24.26 ± 2.56^{a}	20.80 ± 5.00^{a}	11.30 ± 1.77^{ab}	10.00 ± 1.31^{abc}	0.85 ± 0.18^{abc}	27.51 ± 5.53^{ab}	5.76 ± 0.89^{fg}
7	29.13 ± 5.53^{ab}	61.67 ± 12.34^{bc}	23.55 ± 0.66^{abc}	18.33 ± 4.19^{a}	10.53 ± 1.12^{bc}	9.24 ± 1.01^{abc}	0.74 ± 0.06^{bcd}	23.69 ± 3.59^{abc}	9.71 ± 1.64^{abc}
8	27.03 ± 3.22^{ab}	71.31 ± 6.99^{abc}	21.00 ± 1.79^{abcd}	15.84 ± 2.74^{ab}	11.13 ± 0.60^{ab}	9.80 ± 0.23^{abc}	0.70 ± 0.04^{cde}	21.51 ± 1.98^{abc}	7.59 ± 1.34^{cdef}
9	31.20 ± 3.49^{a}	69.11 ± 12.28^{abc}	23.18 ± 1.05^{abcd}	18.89 ± 4.47^{a}	10.83 ± 0.29^{ab}	9.62 ± 0.37^{abc}	0.73 ± 0.06^{bcd}	22.28 ± 0.93^{abc}	9.18 ± 0.63^{abcd}
10	27.00 ± 7.67^{ab}	65.11 ± 14.28^{abc}	20.65 ± 2.76^{bcd}	16.68 ± 5.64^{ab}	9.80 ± 0.89^{bc}	9.37 ± 1.27^{abc}	0.73 ± 0.10^{bcd}	21.22 ± 2.39^{abc}	8.77 ± 1.44^{abcde}
11	33.00 ± 4.30^{a}	72.04 ± 8.61^{abc}	23.66 ± 2.26^{abc}	20.98 ± 6.81^{a}	10.83 ± 1.44^{ab}	9.67 ± 1.22^{abc}	0.71 ± 0.22^{cde}	22.54 ± 7.82^{abc}	9.97 ± 1.28^{ab}
12	26.07 ± 3.65^{ab}	66.93 ± 5.88^{abc}	23.97 ± 1.41^{ab}	20.20 ± 3.08^{a}	11.03 ± 1.37^{ab}	9.86 ± 1.30^{abc}	0.98 ± 0.14^{a}	24.77 ± 9.36^{abc}	10.66 ± 1.69^{a}
13	27.93 ± 6.36^{ab}	68.15 ± 6.59^{abc}	20.23 ± 1.68^{cd}	15.53 ± 3.28^{ab}	9.81 ± 1.22^{bc}	8.97 ± 0.95^{bc}	0.65 ± 0.09^{de}	20.07 ± 2.84^{c}	9.63 ± 0.43^{abc}
14	27.80 ± 9.45^{ab}	69.92 ± 19.92^{abc}	23.31 ± 2.65^{abc}	17.78 ± 7.59^{ab}	11.25 ± 1.34^{ab}	9.89 ± 1.05^{abc}	0.73 ± 0.06^{bcd}	21.75 ± 2.08^{abc}	7.45 ± 1.42^{def}
15	27.00 ± 6.08^{ab}	76.12 ± 11.37^{ab}	23.42 ± 2.91^{abc}	19.03 ± 6.60^{a}	11.28 ± 0.83^{ab}	9.91 ± 0.53^{abc}	0.85 ± 0.16^{abc}	28.04 ± 4.54^{a}	8.25 ± 1.38^{abcde}
16	27.33 ± 5.07^{ab}	65.80 ± 8.79^{abc}	23.71 ± 1.83^{abc}	15.32 ± 3.24^{ab}	10.54 ± 0.21^{bc}	9.95 ± 0.62^{abc}	0.71 ± 0.06^{cde}	23.20 ± 2.86^{abc}	9.58 ± 1.48^{abcd}
17	31.73 ± 2.74^{a}	68.37 ± 7.25^{abc}	22.68 ± 2.28^{abcd}	17.83 ± 3.25^{ab}	10.44 ± 0.86^{bc}	9.42 ± 0.80^{abc}	0.65 ± 0.11^{de}	20.96 ± 6.51^{bc}	8.73 ± 0.54^{abcde}
18	27.73 ± 2.54^{ab}	72.75 ± 12.21^{ab}	22.60 ± 1.82^{abcd}	18.45 ± 1.67^{a}	11.16 ± 1.17^{ab}	10.06 ± 0.91^{ab}	0.81 ± 0.12^{abcd}	24.81 ± 4.28^{abc}	6.77 ± 1.04^{efg}
19	29.87 ± 4.13^{ab}	62.37 ± 10.17^{abc}	21.86 ± 1.90^{abcd}	16.76 ± 5.35^{ab}	10.21 ± 0.57^{bc}	9.28 ± 0.63^{abc}	0.69 ± 0.16^{cde}	21.98 ± 3.22^{abc}	10.21 ± 3.24^{ab}

Different letters in columns are significantly different using one-way ANOVA and Duncan's multiple range test ($P < 0.05$)

Method validation

The UPLC-UV method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. Calibration curves were constructed with eight different concentrations from the following concentration ranges: 6.25–800 μg/mL for three compounds (schisandrin, gomisin A and gomisin N). LOD and LOQ under the present chromatographic conditions were determined at a signal-to-noise ratio 3.3 and 10, respectively. The intra- and inter-day precision (%) were determined by analyzing three replicates of three different concentrations within 1 day or 3 sequential day. The intra- and inter-day precisions were expressed as the relative standard deviation (RSD). Accuracy (%) was evaluated using a recovery assay which was carried out by analyzing the peak areas of standard stock solutions spiked fruits extracts and by analyzing peak areas of fruits extracts with no standard stock solutions added. Each sample was analyzed in triplicate at three different concentrations and expressed as a mean value.

Statistical and multivariate analysis

Statistical analysis was performed using SPSS software (Statistical Package for the Social Science, Version 26, IBM SPSS statistics, IL, USA) and data were expressed as means ± standard deviation (SD). Statistical analyses of the results were performed at a 5% significance level. Differences between the means of individual groups were analyzed by one-way analysis of variance (ANOVA), followed by the Duncan's multiple-range test. The correlation between fruit growth characteristics and marker compounds of *S. chinensis* was confirmed by Pearson's correlation coefficient.

Results and discussion

UPLC-UV method validation

To efficiently identify and quantify the three marker compounds, UPLC-UV method was validated. Linearity was examined with eight different concentrations of the three marker compounds of *S. chinensis* using calibration curves from the linear regression analysis. The three marker compounds showed a good linearity ($r^2 > 0.9998$) within the tested range and LOD and LOQ values ranged from 0.003 to 0.02 μg/mL and 0.01 to 0.07 μg/mL, respectively (Table 2). The values of LOD and LOQ shows that established UPLC-UV method was sufficiently sensitive for determination of lignans in *S. chinensis*. Intra- and inter-day variations were selected to determine the precision of the method. Precision values of intra- and inter-day were appropriate as 0.14 to 0.52% and 0.23 to 2.50%, respectively (Table 3). The average recoveries of the three marker compounds were 98.4 to 101.68% (Table 4), and the RSD was around 2% as a satisfactory value [22].

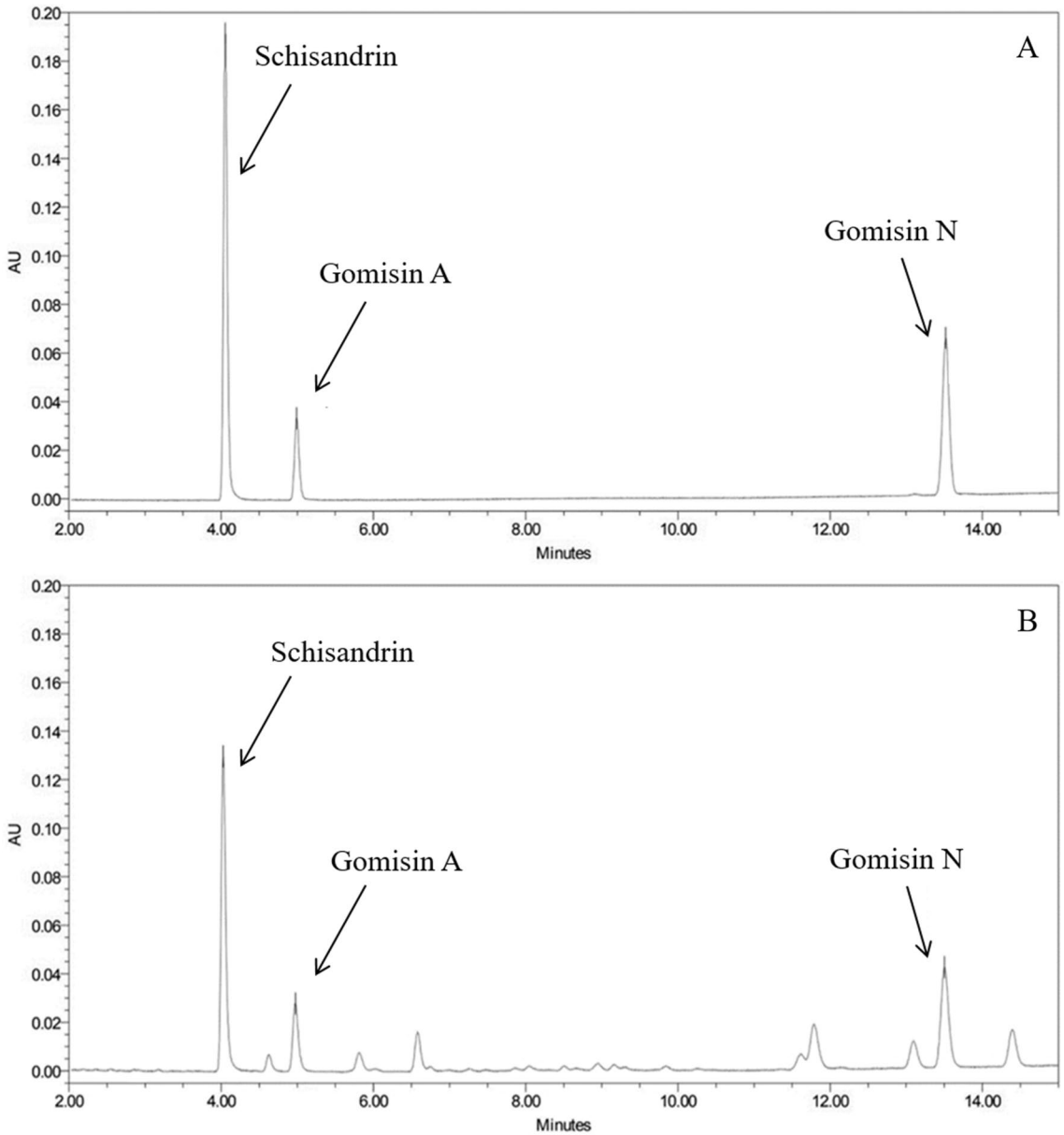

Fig. 2 UPLC chromatograms of *Schisandra chinensis* extracts; **A** standard mixture; **B** region 5 sample extract

As a result, the reproducibility and reliability of the analytical method were verified by method validation through analysis of linearity, LOD, LOQ, precision, and accuracy.

Growth characteristics

The growth data of *S. chinensis* fruits are shown in Table 5. A number of fruits per fruit bunch were 26.07 ± 3.65 to 33.00 ± 4.30. Length, width, and fresh weight of fruit bunch were 56.79 ± 9.81 to 77.61 ± 12.08 mm, 19.70 ± 2.13 to 24.26 ± 2.56 mm, 20.98 ± 6.81 to 11.41 ± 3.43 g, respectively. In addition, length, width, fresh weight of fruit, and fresh weight of 30 fruits were 9.27 ± 0.68 to 12.31 ± 0.39 mm, 8.67 ± 0.67 to 10.58 ± 0.33 mm, 0.54 ± 0.08 to 0.98 ± 0.14 g, 18.00 ± 1.54 to 28.04 ± 4.54 g, respectively. Sugar contents of fruits were 5.33 ± 1.16 to 10.66 ± 1.69 Brix°. It was found that there was a positive correlation between fruit size and bunch size, but there was no significant correlation between fruit size and sugar contents (data not shown). The cultivation region 11 was confirmed as the place with highest number of fruits per fruit bunch, and the

Table 6 Three marker compounds composition of *Schisandra chinensis* in 19 different cultivation regions

Cultivation regions	Schisandrin (%)	Gomisin A (%)	Gomisin N (%)	Total (%)
1	0.42 ± 0.04^{g}	0.41 ± 0.05^{ab}	0.29 ± 0.02^{fgh}	1.12 ± 0.06^{cdef}
2	0.41 ± 0.05^{g}	0.25 ± 0.22^{cde}	0.24 ± 0.08^{gh}	0.90 ± 0.24^{fg}
3	0.46 ± 0.10^{fg}	0.27 ± 0.08^{bcde}	0.21 ± 0.13^{h}	0.94 ± 0.20^{efg}
4	0.53 ± 0.14^{cdefg}	0.19 ± 0.09^{def}	0.39 ± 0.14^{bcdef}	1.11 ± 0.30^{cdef}
5	0.76 ± 0.04^{a}	0.45 ± 0.10^{a}	0.49 ± 0.02^{ab}	1.69 ± 0.16^{a}
6	0.43 ± 0.05^{fg}	0.33 ± 0.05^{abcd}	0.31 ± 0.04^{efgh}	1.07 ± 0.07^{defg}
7	0.46 ± 0.07^{g}	0.16 ± 0.03^{ef}	0.32 ± 0.05^{defgh}	0.95 ± 0.12^{efg}
8	0.71 ± 0.12^{ab}	0.33 ± 0.10^{abcd}	0.42 ± 0.05^{bcde}	1.46 ± 0.22^{ab}
9	0.67 ± 0.13^{abc}	0.30 ± 0.08^{bcde}	0.38 ± 0.07^{bcdef}	1.35 ± 0.17^{bcd}
10	0.49 ± 0.14^{fg}	0.36 ± 0.16^{abc}	0.46 ± 0.06^{abc}	1.31 ± 0.19^{bcd}
11	0.38 ± 0.12^{g}	0.07 ± 0.04^{f}	0.37 ± 0.06^{bcdefg}	0.82 ± 0.13^{g}
12	0.53 ± 0.08^{cdefg}	0.27 ± 0.05^{bcde}	0.35 ± 0.06^{cdefg}	1.15 ± 0.16^{cdef}
13	0.52 ± 0.12^{defg}	0.29 ± 0.13^{bcde}	0.57 ± 0.19^{a}	1.38 ± 0.34^{bc}
14	0.64 ± 0.14^{abcde}	0.37 ± 0.12^{abc}	0.45 ± 0.10^{bcd}	1.46 ± 0.31^{ab}
15	0.52 ± 0.04^{cdefg}	0.30 ± 0.06^{bcde}	0.33 ± 0.05^{defgh}	1.15 ± 0.12^{cdef}
16	0.49 ± 0.05^{efg}	0.26 ± 0.02^{cde}	0.42 ± 0.06^{bcdef}	1.16 ± 0.08^{cdef}
17	0.52 ± 0.10^{defg}	0.27 ± 0.06^{bcde}	0.37 ± 0.07^{bcdef}	1.15 ± 0.13^{cdef}
18	0.66 ± 0.07^{abcd}	0.24 ± 0.06^{cde}	0.40 ± 0.08^{bcdef}	1.30 ± 0.18^{bcd}
19	0.58 ± 0.18^{bcdef}	0.25 ± 0.11^{cde}	0.40 ± 0.10^{bcdef}	1.23 ± 0.29^{bcde}

Different letters in columns are significantly different using one-way ANOVA and Duncan's multiple range test ($P < 0.05$)

Table 7 Pearson's correlation coefficient between fruit growth characteristics and marker compounds of *Schisandra chinensis*

	Correlation coefficient (r)[a]								
	Number of fruits per fruit bunch	Length of fruit bunch	Width of fruit bunch	Fresh weight of fruit bunch	Length of fruit	Width of fruit	Fresh weight of fruit	Fresh weight of 30 fruits	Sugar contents of fruit
Schisandrin	-0.303^{**} (0.003)	-0.262^{*} (0.010)	-0.318^{**} (0.002)	-0.476^{**} (0.000)	−0.238 (0.020)	−0.184 (0.074)	-0.343^{**} (0.001)	-0.370^{**} (0.000)	-0.469^{**} (0.000)
Gomisin A	−0.182 (0.078)	−0.198 (0.054)	-0.225^{*} (0.028)	-0.257^{*} (0.012)	−0.135 (0.192)	−0.140 (0.176)	−0.158 (0.127)	−0.188 (0.068)	-0.302^{**} (0.003)
Gomisin N	−0.129 (0.214)	−0.187 (0.069)	-0.405^{**} (0.000)	-0.364^{**} (0.000)	-0.275^{**} (0.007)	−0.165 (0.110)	-0.369^{**} (0.000)	-0.407^{**} (0.000)	-0.209^{*} (0.042)
Total	-0.283^{**} (0.005)	-0.294^{*} (0.004)	-0.422^{**} (0.000)	-0.498^{**} (0.000)	-0.290^{**} (0.004)	-0.220^{*} (0.032)	-0.390^{**} (0.000)	-0.432^{**} (0.000)	-0.452^{**} (0.000)

[a] Correlation coefficient (r) written is significantly correlated between the variables compared. Positive values denote positive correlation and negative values denote negative correlation.

Values in bracket means p value

$^{**}p < 0.01$

$^{*}p < 0.05$

diameter of the fruit was confirmed to be the largest in 2 cultivation regions, and the fresh weight of fruit was confirmed to be the heaviest in 12 cultivation regions. Also, the sugar contents of fruits was confirmed to be high in no.12 and 2 cultivation regions. It was also reported that number of fruit bunch and weight of 100 ea fruits of *S. chinensis* were 2.0 ± 1.4 to 31.0 ± 8.5 and 4.5 ± 0.7 to 87.0 ± 28.3 g, respectively [17]. Compared to our data, the fruit quality data showed some differences in the minimum value, but generally similar trends. This difference in fruits by cultivation region may be due to soil and meteorological factors in the cultivation regions. It was reported that environmental factors such as altitude,

light, and precipitation in cultivation regions and soil properties such as soil pH, nitrogen content, irrigation, and drainage affect plant growth [23–25].

Quantitative analysis of marker compounds

The UPLC-UV method was applied to the 95 samples of *S. chinensis*. The three marker compounds were identified by comparing retention time and UV spectra chromatograms of the peaks with those of the standards in UPLC chromatogram. Three marker compounds, schisandrin, gomisin A and gomisin N were detected at retention time of 4.0, 4.9 and 13.5 min, respectively (Fig. 2). The samples contained schisandrin from 0.41 ± 0.05 to 0.76 ± 0.04%, gomisin A from 0.07 ± 0.04 to 0.45 ± 0.10% and gomisin N from 0.21 ± 0.13 to 0.49 ± 0.02% (Table 6). The most schisandrin contents (0.76 ± 0.04%) was confirmed to be cultivation region 5, and gomisin A (0.41 ± 0.05%) was cultivation region 1, gomisin N (0.57 ± 0.19%) was cultivation region 13. Total cotent of three marker compounds was confirmed to be cultivation reion 5 (1.69 ± 0.16%). It was also noted that the content of eleven lignans in *S. chinensis* was different depending on the region in China [26].

Although there was a difference in the ratio of marker compounds in 19 regions, the sum of the three marker compounds was 0.82 to 1.46%, exceeding the 0.7% standard of Korean Pharmacopoeia (Table 6) [21]. These results were similar to those reported in the study of Hwang et al. [17], where the sum of three marker compounds was 0.8 to 1.2% in 26 regions.

Correlation between growth characteristics and marker compounds of *S. chinensis* fruit

The results of correlation analysis between growth characteristics and three marker compounds of *S. chinensis* fruit are presented in Table 7. The contents of three marker compounds, schisandrin, gomisin A and gomisin N were found to have a negative correlation with most of the fruit growth characteristics, and in particular, had a significantly negative correlation with fresh weight and sugar contents of fruits. Fresh weight of fruits is significantly correlated with schisandrin ($r = -0.343$, $p < 0.01$), gomisin N ($r = -0.369$, $p < 0.01$) and total marker compounds ($r = -0.390$, $p < 0.01$). Sugar contents of fruits are significantly correlated with schisandrin ($r = -0.469$, $p < 0.01$), gomisin A ($r = -0.302$, $p < 0.01$), gomisin N ($r = -0.209$, $p < 0.05$) and total marker compounds ($r = -0.452$, $p < 0.01$). Cultivation methods only for growth can cause a decrease in the compound contents. Park et al. [27] reported that there was a negative correlation ($r = -0.437$) between dry weight of root and total contents of active compound (nodakenin, decursin, and decursinol angelate) in *Angelica gigas* Nakai. And they mentioned that various non-biological stresses can affect the growth of plant or specific functional compounds. Also, there was a clear change by lighting in the cultivation of lettuce, with the red light being the most effective on growth and the blue light being the most effective on the biosynthesis of secondary metabolites [28].

Therefore, future studies need to focus on the correlation between soil physicochemical properties, meteorological environment of the cultivation region and marker compounds of *S. chinensis* fruit.

In this study, simultaneous UPLC analysis conditions of three marker compounds, schisandrin, gomisin A and gomisin N of *S. chinensis* were established. The growth characteristics and lignans contents of *S. chinensis* fruit in cultivation regions across the South Korea were investigated. Correlation between growth characteristics and contents of lignans in *S. chinensis* fruits by region was also analyzed. There was a significantly negative correlation between lignans contents and growth characteristics such as fruit size, weight. These results can be used to study the standard cultivation manual of *S. chinensis* fruits for medicinal purposes. However, studies on how the cultivation environment affects the marker compounds of *S. chinensis* fruit are still necessary.

Abbreviations

HPLC: High-performance liquid chromatography; LOD: Limit of detection; LOQ: Limit of quantification; RSD: Relative standard deviation; SD: Standard deviation; UPLC: Ultra-performance liquid chromatography.

Acknowledgements

This work was supported by the research of National Institute of Forest Science (NIFoS) (Project No. FP0400-2022-02-2022).

Author contributions

DHL: formal analysis; investigation; software; writing-original draft. YP: Design; collecting; project administration. JHJ: Collecting; formal analysis; validation. YS: Collecting. JAK: collecting. S-YL: Writing-review and editing. H-JP: Conceptualization; supervision; writing-review and editing. All authors read and approved the final manuscript.

Funding

National Institute of Forest Science (NIFoS), Republic of Korea (Project No. FP0400-2022-02-2022) funded this study.

Availability of data and materials

The data and materials used in this study are available under permission from the corresponding author on reasonable request.

Declarations

Competing interests

There are no conflicts of interest to declare.

Author details

[1]Forest Medicinal Resources Research Center, National Institute of Forest Science, Yeongju 36040, South Korea. [2]Department of Forest Bioresources, National Institute of Forest Science, Suwon 16631, South Korea.

Received: 8 August 2022 Accepted: 31 October 2022
Published online: 17 November 2022

References

1. Lebedev AA (1971) Limonnik (*Schisandra chinensis*). Medicina Publishing House, Tashkent
2. Atsumi T, Yokoyama Y, Yokogawa T, Makino T, Ohtsuka I (2019) Effects of crushed *Schisandra* fruit on the content of lignans in kampo decoction. J Nat Med 73(2):381–387
3. Kim M, Sung H, Park J, Sohn H (2017) Evaluation of anti-oxidant, anti-microbial and anti-thrombosis activities of fruit, seed and pomace of *Schizandra chinensis* Baillon. J Life Sci 27(2):131–138
4. Lu Y, Chen D (2009) Analysis of *Schisandra chinensis* and *Schisandra sphenanthera*. J Chromatogr A 1216(11):1980–1990
5. Guo LY, Hung TM, Bae KH, Shin EM, Zhou HY, Hong YN, Kang SS, Kim HP, Kim YS (2008) Anti-inflammatory effects of schisandrin isolated from the fruit of *Schisandra chinensis* Baill. Eur J Pharmacol 591:293–299
6. Li X, Zhao B, Liu G, Xin W (1990) Scavenging effects on active oxygen radicals by schizandrin with different structures and configurations. Free Radic Biol Med 9(2):99–104
7. Kim Y, Ha N, Han S, Jeon J, Hwang M, Im Y, Lee SY, Chae S, Kim M (2013) Confirmation of schizandrin as a marker compound in Jangsu omija powder. J Korean Soc Food Sci Nutr 42(2):244–248
8. Wang R, Kong J, Wang D, Lien LL, Lien EJ (2007) A survey of chinese herbal ingredients with liver protection activities. Chin Med 2:5
9. Tang S, He R, Huang T, Wang C, Cao Y, Zhang Y, Kurihara H (2011) The protective effect of *Schisandra* lignans on stress-evoked hepatic metastases of P815 tumor cells in restraint mice. J Ethnopharmacol 134:141–146
10. Duan Y, Cao J, Wen R, Yang G, Pu J, Sun H, Xiao W, Li G (2011) Dibenzocyclooctadien lignans from *Schisandra neglecta* and their andti-HIV-1 activities. J Asian Nat Prod Res 13(7):592–598
11. Nishiyama N, Chu P, Saito H (1996) An herbal prescription, S-113m, consisting of biota, ginseng and schizandra, improves leraning performance in senescene accelerated mouse. Biol Pharm Bull 19(3):388–393
12. Panossian A, Wikman G (2008) Pharmacology of *Schisandra chinensis* Bail an overview of Russian research and uses in medicine. J Ethnopharmacol 118(2):183–212
13. Deng X, Chen X, Cheng W, Shen Z, Bi K (2008) Simultaneous LC-MS quantification of 15 lignans in *Schisandra* chinensis (Turcz) Baill fruit. Chromatographia 67(7):559–566
14. Saleem M, Kim HJ, Ali MS, Lee YS (2005) An update on bioactive plant lignans. Nat Prod Rep 22(6):696–716
15. Min H, Park E, Hong J, Kang Y, Kim S, Chung H, Woo E, Hung TM, Youn UJ, Kim YS, Kang SS, Bae K, Lee SK (2008) Antiproliferative effects of dibenzocyclooctadiene lignans isolated from *Schisandra chinensis* in human cancer cells. Bioorganic Med Chem Lett 18(2):523–526
16. Choi S, Lee Y, Wang RB, Kim H, Yoon D, Lee DY, Kim Y, Baek N (2020) Dibenzocyclooctadiene lignans from the fruits of *Schisandra chinensis* and their cytotoxicity on human cancer cell lines. Appl Biol Chem 63(1):39
17. Hwang BS, Kim G, Park YH, Hwan IG (2019) Method validation and quantification of lignanas in the fruits of Korean omija (*Schisandra chinensis*) by cultivation region. J Korean Soc Food Sci Nutr 48(10):1105–1111 (**(in Korean)**)
18. Kim JH, Lee BG, Choi EY (2017) Effects of vine induction method on the growth and fruit yield in Korean schisandra. Korean J Med Crop Sci 25(2):83–88 (**(in Korean)**)
19. Kandimalla R, Das M, Barge SR, Sarma PP, Koiri DJ, Devi A, Karki AK, Kurma A, Devi R, Pal BC, Samanta TNC, SK, (2020) Variation in biosynthesis of an effective anticancer secondary metabolite, mahanine in *Murraya koenigii*, conditional on soil physicochemistry and weather suitability. Sci Rep 10(1):1–11
20. Radić S, Vujčić V, Glogoški M, Radić-Stojković M (2016) Influence of pH and plant growth regulators on secondary metabolite production and antioxidant activity of *Stevia rebaudiana* (Bert). Period Biol 118(1):9–19
21. Ministry of Food and Drug Safety (2022) The Korean Pharmacopoeia 12th ed. The KFDA notification No. 2022–10: Subparagraph 4 of Article 2. Chungju, Korea. p 75
22. Marson BM, Concentino V, Junkert AM, Fachi MM, Vilhena RO, Pontarolo R (2020) Validation of analytical methods in a pharmaceutical quality system: an overview focused on HPLC methods. Quim nova 43(8):1190–1203
23. Seo Y, Kim J, Kim J, Kim Y, Ahn Y, Cha S (2014) Effects of fertiliazation and co-application of compost tea on fruit growth and accumulation of anthocyanin in omija (*Schisandra chinensis* Baillon). Korean J Soil Sci Fert 47(6):547–552
24. Houtier Y, Randin CF, Stöcklin J, Guisan A (2009) Changes in reproductive investiment with altitude in an alpine plant. J Plant Ecol 2(3):125–134
25. Kirnak H, Higgs D, Kaya C, Tas I (2005) Effects of irrigation and nitrogen rates on growth, yield, and quality of muskmelon in semiarid regions. J Plant Nutr 28(4):621–638
26. Lu T-L, Hu J-Y, Mao C-Q, Wu Y, Yin F-Z, Cai B-C (2012) Quality analysis of raw and processed *schisandra chinensis* fructus by simultaneous determination of eleven bioactive lignans using RP-HPLC method. J Food Drug Anal 20(4):922–929
27. Park Y, Park PS, Jeong DH, Sim S, Kim N, Park H, Jeon KS, Um Y, Kim M (2020) The charateristics of the growth and the active compounds of *Angelica gigas* Nakai in cultivation sites. Plants 9(7):823
28. Son K, Lee J, Oh Y, Kim D, Oh M (2017) Growth and bioactive compound synthesis in cultivated lettuce subject to light-quality changes. HortScience 52(4):584–591

Publisher's Note