Using UHPLC and UV-vis Fingerprint Method to Evaluate Substitutes for *Swertia mileensis*: An Endangered Medicinal Plant

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ABSTRACT

Background: Millions of people are killed by viral hepatitis every year in the world, whereas many relevant medicines are too expensive to purchase. *Swertia mileensis*, a medicinal plant for hepatitis in the system of traditional Chinese medicine, has been vanishing gradually because of overexploitation. **Objective**: To find substitutes of *S. mileensis* and reduce the cost of purchasing drugs for hepatitis patients, the similarity of phytochemical constituents between *S. mileensis* and other three *Swertia* species was compared. **Materials and Methods:** Both ultra high performance liquid chromatographies and ultraviolet-vis fingerprints of four *Swertia* species were developed. Methanol extracts of the stems and leaves were used as samples to establish the fingerprint. The calibration curve was drawn for quantitative analysis of swertiamarin. The data of ultra high performance liquid chromatographies were evaluated statistically using similarity analysis and principal component analysis. **Results**: The result shows a significant difference at area of 204–290 nm in the ultraviolet fingerprint. Swertiamarin, the only one common peak, was defined in chromatographic fingerprints of four *Swertia* species. The quantitative analysis suggested that the highest concentration of swertiamarin is in *S. davidii*. The similarity indexes between different samples were almost under 0.60. In the principal component analysis, separate points not only represent the distinction among different species, but also perform chemical discrepancies in content between stems and leaves of one same species. **Conclusions**: *S. angustifolia*, *S. davidii*, and *S. punicea* are not suitable as substitutes of *S. mileensis* because of their remarkable differences in entirety and local part. In order to address issues about substitutes and high cost of purchasing drugs, more studies need to undertake.

Key words: Fingerprint, *Swertia mileensis*, ultra high performance liquid chromatographies, ultraviolet-vis

SUMMARY

- The UHPLC fingerprint method indicated the significant difference on chemical ingredients in four plants from *Swertia*.
- Swertiamarin is the unique common compounds for four plants, which exist are in leaves of *S. davidii* with the highest content.
- The obvious diversity in four plants was displayed from comprehensive point of view though similarity assay and PCA analysis.
- The UV fingerprint method offsets the defect that the UHPLC fingerprint reflected messages of secoiridoid glycosides only.
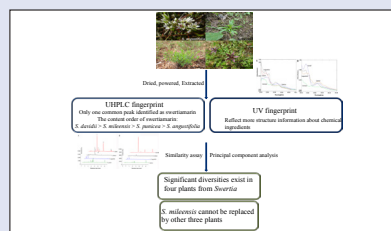
Abbreviation used: UHPLC: Ultra high performance liquid chromatography, UV-vis: Ultraviolet-vis, HBV: Anti-hepatitis virus, DNA: Deoxyribonucleic acid, PCA: Principal component analysis, D-GalN: D-Galactosamine, BCG: Bacille Calmette-Guerin, LPS: Lipopolysaccharide

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INTRODUCTION

According to World Health Organization, about two million people are infected with viral hepatitis from unsafe injection every year, especially hepatitis B and C, which together cause roughly 80% of all liver cancer deaths and kill close to 1.4 million people.[1] Viral hepatitis, featured on silent or benign nature in early stages, is induced by one of the five hepatitis viruses involved in types A, B, C, D, and E.[2] African region is considered as the highest prevalence of viral hepatitis in the world, as well as some areas shrouded in the shadow, such as regions of Latin America and the Caribbean, Eastern Mediterranean, Europe, South-East Asia, and Western Pacific.[3] The spread of hepatitis disease has been decreased, which is attributed to persistent improvement of sanitary and living conditions, whereas it causes reductions of acquisitive immunity and universal vaccination. Furthermore, although the medicines for treatment of viral hepatitis efficiently are sold in markets, few patients can afford them because of their high prices. Compared with those famous medicines, some inexpensive and effective herbal drugs, such as

Swertia mileensis, may be better choices for hepatitis patients living in developing countries.

S. mileensis T. N. Ho et W. L. Shi (Qingyedan in Chinese), belonging to Gentianaceae family, is distributed in the southern part of Yunnan province in China. It is available in thick patch of grass on versants at an altitude of 1300–1650 m.[4] *S. mileensis* was documented in the

Chinese Pharmacopoeia due to its potent hepatoprotective activity.[5] Geng *et al.*[6,7] found that swerilactones in *S. mileensis* exhibit potent anti-hepatitis B virus (HBV) activity through inhibiting HBV deoxyribonucleic acid (DNA) replication or against the secretion of hepatitis B and e surface antigen, which show the potential to treat viral hepatitis. So far, researchers have extracted and isolated many types of constituents from *S. mileensis*, mainly including secoiridoids, xanthones, monoterpens, and lactones, which are responsible for therapeutic properties.[7-10] Despite all this, *S. mileensis* has not received the attention that it deserves from national community. In past years, the resources of *S. mileensis* have vanished gradually because of destructive collection, for which Yang *et al.*,[11] explored the sustainable utilization of this plant through investigating its phenological phase, biological characteristics, and ecological environment. What is really exciting is that modern pharmacological studies have demonstrated that certain species of the genus *Swertia* also exhibit a variety of bioactivities, such as anti-HBV, antidiabetic, antimalarial, and antibacterial activities.[12-16] Coefficient of similarity is an indicator to measure the level of similarity in different samples, which can reflect similarity degree between two members directly.[17] The traditional Chinese medicine, the precious of people on China, characterized by the Theory of Whole View[18] that means, for drug applications, synergistic effects and multiple targets of many compounds or dissociating products of these compounds may happen in the therapeutic procedure rather than a monomer constituent.[19, 20] In other words the holistic similarity degree of phytochemical constituents was related positively with the therapeutic reactions in *Swertia* Species when they were used to cure same ailments. Hence, in order to find substitutes of *S. mileensis* and reduce the cost of purchasing drugs, it is urgent to assess integrally the similarity degree of phytochemical constituents between *S. mileensis* and other members of the genus *Swertia* through the fingerprint method.

During the past few years, researchers have developed some works for researches of chemical components in the genus *Swertia*.[21] Geng *et al.*[22] identified the structures of four secoiridoids in *S. mileensis* using diverse spectroscopy methods. Eight compounds were isolated from aerial parts of *S. angustifolia* by Zhu *et al.*,[23] four of which were gained firstly. While there are a number of peer-reviewed publications reporting content determination of chemical compounds in a single species,[24, 25] the information about contrasts in different *Swertia* species is limited.[26] Therefore, to evaluate the overall similarity of the genus *Swertia*, more interspecific studies are still necessary to be carried out.

A comprehensive and quantifiable identification method of chromatographic fingerprint which been regarded as the first choice by World Health Organization,[27] is able to display chemical information of herbal medicines with chromatogram, spectrograms, and other graphs by analytical and chemical techniques.[28] The ultra high performance liquid chromatographies (UHPLCs), a common instrument for quantification of components, possess two merits which are short total run time and simultaneous determination of compounds. Inoue *et al.*[29] successfully evaluate gardenia yellow based on UHPLC. The goal of identifying crude drugs could be achieved with the ultraviolet-visible absorption spectrometry (UV-vis), because chemical constituents from different materials have different unsaturated degree, which causes different peak positions, peak intensities, and shapes of curves.[30] In this paper, in order to obtain holistic similarity evaluation of different species (*S. mileensis*, *S. angustifolia*, *S. davidii*, and *S. punicea*) from *Swertia* genus, an UHPLC combined with the UV-vis fingerprint method will be established. To the best of our knowledge, this study could be considered as the first report on fingerprints development of these four species. This research may become a base stone to study plants of *Sweria* genus further in the future.

MATERIALS AND METHODS

Reagents and materials

The swertiamarin reference standard (purity ≥ 98%) was purchased from Shanghai Shifeng Biological Technology Co., Ltd (Shanghai, China). Methanol and formic acid of HPLC grade were obtained from Thermo Fisher Scientific (USA) and Dikmapure (USA), respectively. Purified water was purchased from Hangzhou Wahaha Group (Hangzhou, China). All other chemicals and reagents were analytical grade. Four batches of plants from the genus *Swertia* were collected in three provinces of China. All of the samples were identified by Dr. Heng-Yu Huang (College of Traditional Chinese Medicine, Yunnan University of Traditional Chinese Medicine, Kunming, China). The information of materials is shown in Table 1.

Instrumentation and analytical conditions

A Shimadzu UPLC system equipped with a degasser, binary gradient pumps, a column oven, an auto sampler and a UV detector, was employed for the chromatography analysis. The UV-vis analysis was built using the binary channel UV-Vis spectrophotometer (Shimadzu, Japan) with the UV spectra of 190–900 nm. Sample solutions of 3 mL were poured into cuvettes for this analysis. Under the UHPLC condition, all of analytes were detected on Shim-pack XR-ODS Ⅲ (150×2.0 mm, 2.2 μm) C_{18} column by gradient elution with the mobile phase of 0.1% formic acid (A) and methanol (B) at the flow rate of 0.35 mL•min^{-1}, which the detective wavelength was set at 240 nm. The gradient program was as follow: 0–1 min, 0–18% B; 1–4.5 min, 18–28% B; 4.5–5.7 min, 28–30% B; 5.7–11.5 min, 30–40% B; 11.5–15.2 min, 40–54% B; 15.2–19.5 min, 54–90% B; 19.5–22.0 min, 90% B; 22.0–25.0 min, 90–18% B. The column temperature and injection volume were set at 40°C and 1μL, respectively. The extracts were stored at 4°C.

Preparation of samples

Dried stems and leaves of *S. angustifolia*, *S. davidii*, *S. punicea*, and *S. mileensis* were powdered respectively as homogeneous size and sifted through a 60 mesh sieve. About 0.025 g of powder was weighed accurately and processed by ultrasonic extraction with 1.5 mL 80% methanol for 30 min. Extraction solutions were filtered through a 0.22 μm membrane filter. The filtrate collected in auto sampler vials was analyzed directly by UHPLC. Methanol and water supplemented 0.1% formic acid were degassed by ultrasonication for 30 min before use to avoid micro bubbles in solutions.

Dried stems and leaves of four species mentioned above were ground into fine power. 0.01 g sample dissolved in 10 mL 100% methanol was extracted ultrasonically for 30 min. The extract was diluted after filtering through a filter paper for UV-vis analysis.

Optimization of UV-vis

Various solvents including methanol, ethanol, petroleum ether, and water were tested. 0.01 g fine power of samples was weighed accurately

Table 1: Information of four batches of plants from the genus *Swertia*

Number	Samples	Places of origin
CJ	Stems of *S. davidii*	Baifusi Town, Enshi, Hubei province, China
QJ	Stems of *S. mileensis*	Mengzi, Yunnan province, China
XJ	Stems of *S. angustifolia*	Xingyi, Guizhou province, China
ZJ	Stems of *S. punicea*	Xingyi, Guizhou province, China
CY	Leaves of *S. davidii*	Baifusi Town, Enshi, Hubei province, China
QY	Leaves of *S. mileensis*	Mengzi, Yunnan province, China
XY	Leaves of *S. angustifolia*	Xingyi, Guizhou province, China
ZY	Leaves of *S. punicea*	Xingyi, Guizhou province, China

to dissolve into these different solvents separately. Then, the ultrasonic extraction of sample solutions maintained 30 min. Subsequently, each 0.5 mL sample supplemented with the 3 mL corresponding solvent was used for UV analysis.

Under the extract condition in the part of solvent optimization, filtrates of samples were obtained. Samples mixed by 0.5 mL filtrates and 3 mL methanol (7-fold) were investigated, as well as samples mixed by 0.5 mL filtrates and 5 mL methanol (11-fold). Then, mixtures were employed for UV analysis.

RESULTS

Method validation and optimization of UV-vis

The standard solution containing swertiamarin was diluted to different concentrations to plot the calibration curve. The calibration curve was gained by plotting peak area (y) versus the concentrations (x, μg•mL^{-1}) of the standard solution. The limit of detection and quantification, signal-to-noise of 3 and 10 were determined by serial dilution of the standard solution using the described conditions. The result is shown in Table 2.

The precision was investigated for the developed method and instruments. The standard solution of swertiamarin was analyzed six times successively for UHPLC and the methanol extract of *S. davidii* for UV. To investigate the repeatability of developed method, six independent samples prepared from *S. davidii* were tested for UHPLC and UV. The prepared sample solutions were stored at room temperature for the stability assay, which

were analyzed at 0, 2, 4, 8, 12, and 24 h for UHPLC and UV, respectively. Satisfactory values of RSD% were obtained. The result is listed in Table 3. Recovery tests were chosen to determine the accuracy of the method. Three different amounts (low, medium, and high spike) of the standard solution of swertiamarin were added into the leave extracts of *S. mileensis*. The recovery rate was calculated using the amount added of standard, and the actual amount (measured amount–original amount) obtained by UHPLC analysis. The result is shown in Table 4.

$$\%R = [(\text{measured amount original amount}) / \text{amount added}] \times 100\%$$

The extraction solvents and dilution multiple were optimized. The result showed that methanol produced the best yield for the four species. Then, the dilution of 7-fold was enough to obtain an available concentration for running process of UV-vis. On the basis of above findings, the best pretreatment was found to be optimal with dilution of 7-fold following methanol extraction. The result of pretreatment is showed in Figure 1.

UV-Vis fingerprint analysis

The UV data were processed by UVProbe software (Shimadzu, Japan). Absorption peak curves of all samples were addressed with 4-point smoothing to suppress the interference from baseline drift and noise.

UV-vis fingerprints of stems and leaves of four different *Swertia* species are showed in Figure 2, respectively. According to two UV fingerprint graphs, the diversity is significant among these four species. Compared with stems, the UV absorbance is remarkably higher in leaves. Both UV
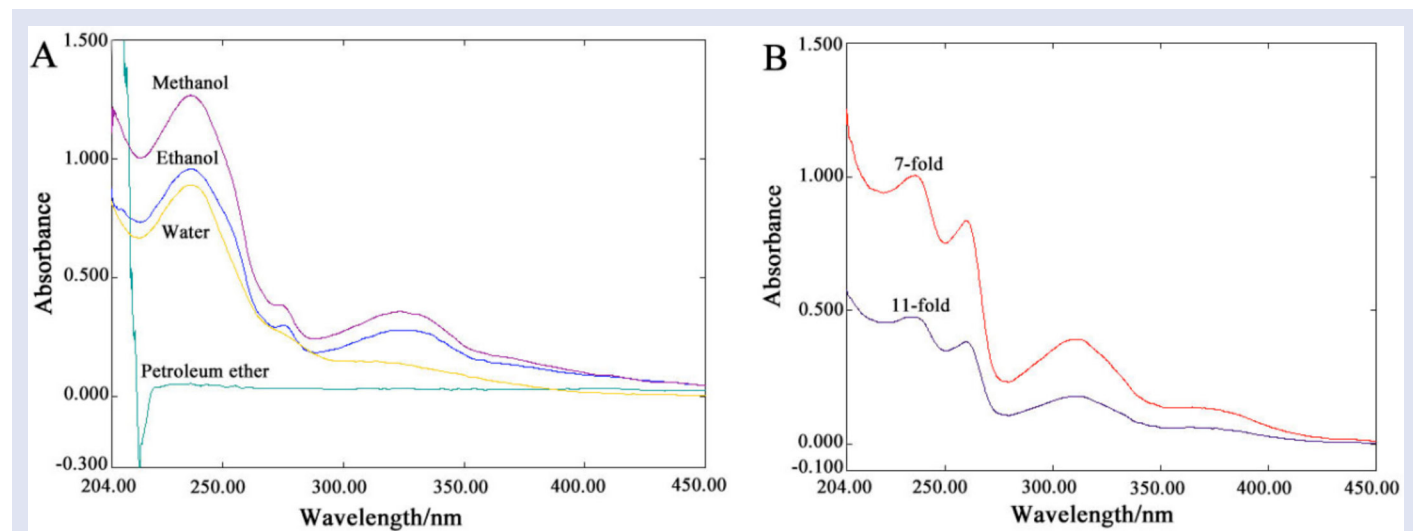

Figure 1: UV-Vis diagrams of optimization of extraction solvents (A) and dilution multiple (B).

Table 2: The calibration curve, related coefficient, linear range, limit of detection, and limit of quantification of swertiamarin

Component	Retention time (min)	Regression equation	R^2	Linear range (μg • mL^{-1})	LOD (μg • mL^{-1})	LOQ (μg • mL^{-1})
Swertiamarin	5.022	Y=2548.34X+24987.9	0.9998	1.79-3200	6.53	19.79

LOD: Limit of detection; LOQ: Limit of quantification

Table 3: Values of RSD% in verification of methods

	Precision/%	Repeatability/%	Stability/%
UHPLC	5.1	0.9	0.6
UV	0.9	4.9	3.2

UHPLC: Ultra high performance liquid chromatographies ; **UV:** Ulraviolet

Table 4: Recovery study of swertiamarin by the developed method

Analyte	Amount added (μg•mL^{-1})	Actual amount (μg•mL^{-1})	Measured amount (μg•mL^{-1})	Recovery%	RSD%
Swertiamarin	104.54645	209.0929	314.6534506	100.97	1.42
	209.09290		417.8512514	101.76	1.56
	313.63935		521.4776926	99.60	0.60

fingerprints of stems and leaves could be divided into two parts, one of which is 204–290 nm and another is 290–450 nm. It is clear in two UV spectrum graphs that characteristic absorption peaks of these four plants were concentrated on the area of 204–290 nm, while their absorption tendencies in the area of 290–450 nm were similar approximately. Comparing two fingerprints of stems and leaves, two organs of one same sample were very close on number and wavelength of peaks but obviously distinct on absorbance. The highest absorbance was seized by *S. angustifolia* in stems at 204–290 nm and in leaves was by *S. davidii*. Following the elaborative observation, some details were found that *S. angustifolia*, *S. davidii*, and *S. punicea* have a common peak at 240 nm, and *S. davidii*, *S. punicea*, and *S. mileensis* have a common peak at 274 nm. Interestingly, the peak at 211 nm involved in stems of *S. punicea* was deficient in its leaves. The only common point between *S. punicea* and *S. mileensis* is the peak at 211 nm. Therefore, the inference we conjectured is that there are diverse prominently in aspects of content and type of chemical compositions because of different UV absorbance and the amount of absorption peaks in part of 204–290 nm.

UHPLC fingerprint analysis

UHPLC fingerprints of stems and leaves of different *Swertia* species have shown their characteristics, which is exhibited in Figure 3. Every sample had some characteristic peaks and showed their fingerprint features. Whatever the species we observed, the amount and type of characteristic peak were consistent in its stems and leaves, except for the intensity. According to the compared analysis of different samples, only one characteristic peak (peak 2) exceeded the baseline obviously in *S. mileensis*, which was defined as swertiamarin through comparing samples with corresponding reference standard. On contrary, eight characteristic peaks (peaks 1–8) were placed above the baseline in *S. davidii*. In addition, 3 (peak 2 and 7) and 5 (peak 2–5 and 7) peaks appeared in *S. angustifolia* and *S. punicea*, respectively. In *S. angustifolia*, intensities of three characteristic peaks were extremely weak so that fused with the baseline almost. So, there were different kinds of characteristic peaks in different species. To sum up, stems and leaves belonged to one species contain similar chemical compounds with different contents. Then, different contents and types of chemical were involved in different
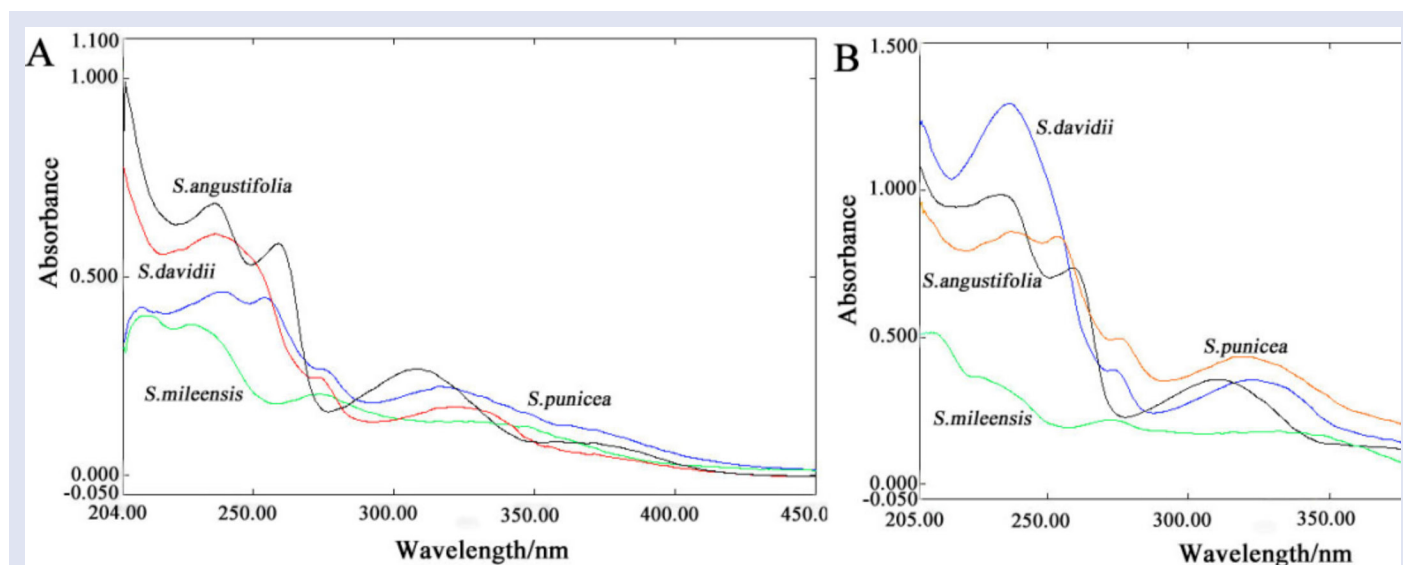

Figure 2: UV fingerprints of stems (A) and leaves (B) from four *Swertia* species.
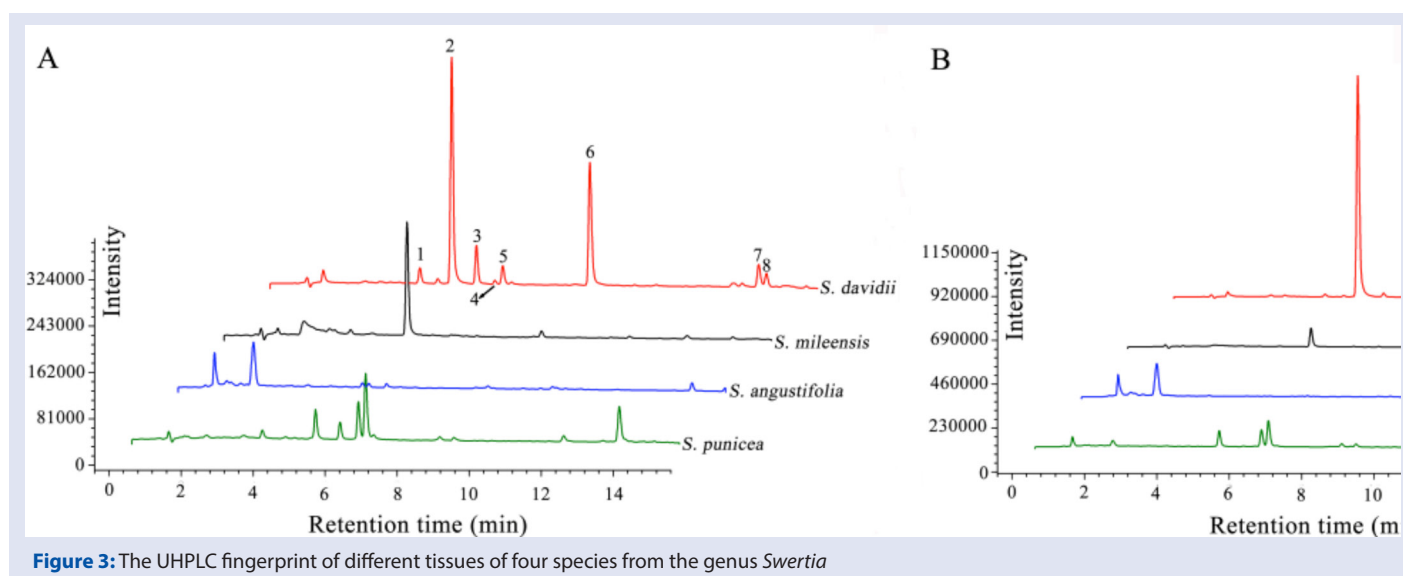

Figure 3: The UHPLC fingerprint of different tissues of four species from the genus *Swertia*

species. The exclusive resemblance between these four species is that they all possessed peak 2. Therefore, the unique common characteristic peak of swertiamarin could be considered as the common constituent of *S. angustifolia*, *S. davidii*, *S. punicea*, and *S. mileensis*.

In order to understand difference among four species further, particularly in the slightly similar part, we chose swertiamarin, an indicator in quality evaluation of *S. mileensis*.[5] to analyze continually. Peak areas were

substituted into the regression equation gained through the calibration curves to calculate contents of each sample. The calculation result is shown in Figure 4. On the basis of quantitative analysis of swertiamarin, the highest content of 159.8212 mg•g^{-1} is in leaves of *S. davidii* and the lowest of 0.3701 mg•g^{-1} is in stems of *S. angustifolia*, whereas the swertiamarin was not detected in leaves of the latter. Both contents of *S. davidii* and *S. punicea* are higher in leaves than in stems, but *S. mileensis*
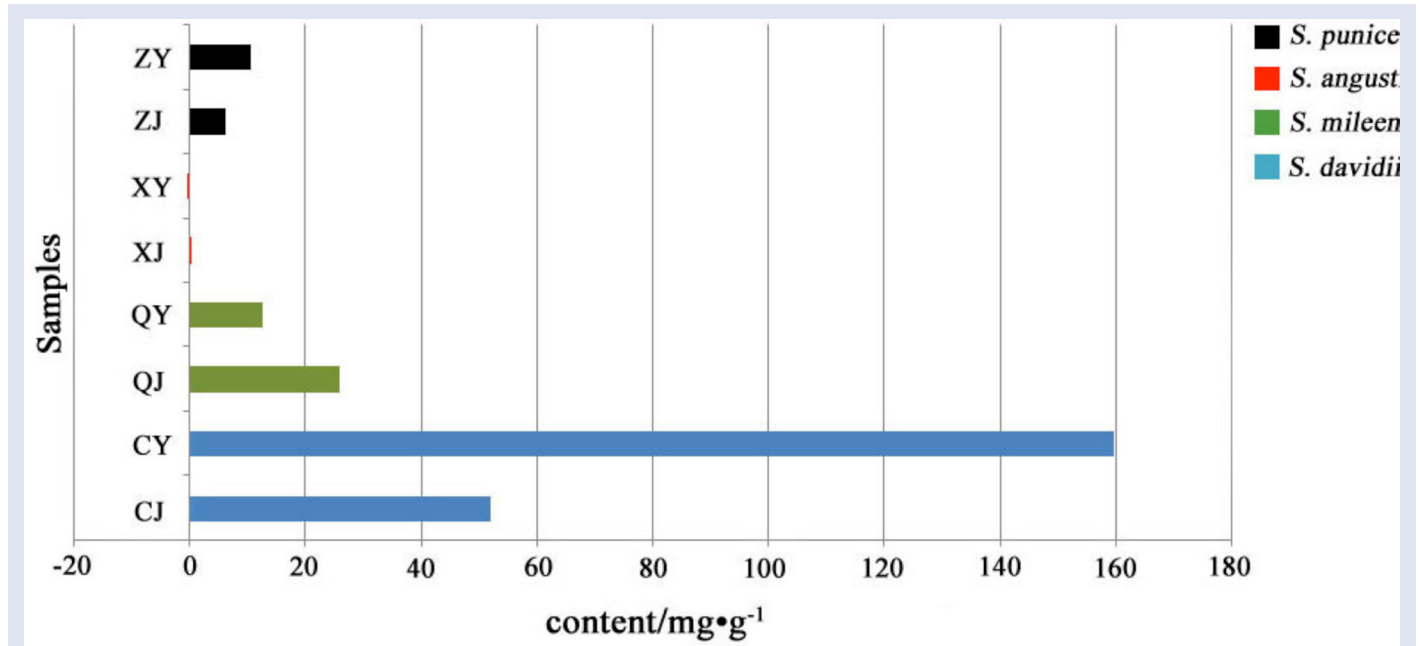

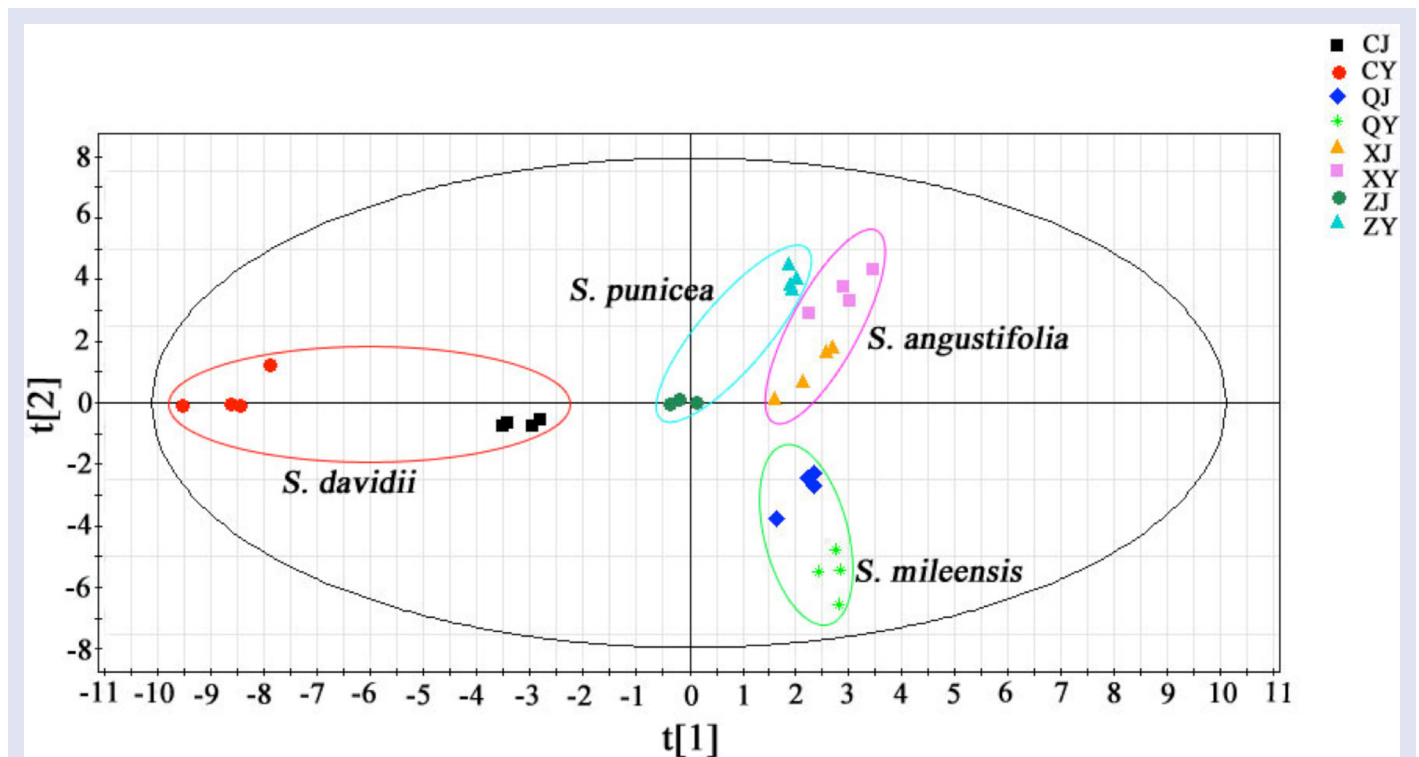**Figure 4:** Concentrations of four *Swertia* species calculated with the calibration curve

Figure 5: The PCA scatter plot of four species from the genus *Swertia*

Table 5: The calculate result of similarity of four species from the genus *Swertia*

	CJ	CY	QJ	QY	XJ	XY	ZJ	ZY	Reference
CJ	1	0.921	0.827	0.813	0.084	0.014	0.409	0.374	0.917
CY	-	1	0.926	0.93	0.086	0.016	0.453	0.475	0.976
QJ	-	-	1	0.936	0.347	0.28	0.318	0.298	0.918
QY	-	-	-	1	0.085	0.014	0.322	0.28	0.882
XJ	-	-	-	-	1	0.99	0.074	0.124	0.234
XY	-	-	-	-	-	1	0.043	0.109	0.168
ZJ	-	-	-	-	-	-	1	0.885	0.578
ZY	-	-	-	-	-	-	-	1	0.593
Reference	-	-	-	-	-	-	-	-	1

and *S. angustifolia* is contrary. The order of swertiamarin content is *S. davidii* > *S. mileensis* > *S. punicea* > *S. angustifolia*.

Similarity assay

Compared with the reference chromatogram generated through mean method, the similarity indexes of stems and leaves from *S. angustifolia*, *S. davidii*, *S. punicea*, and *S. mileensis* were almost under 0.60. This indicated that the differentiation among our eight samples was significant. Nevertheless, through careful observations, there were some unobvious resemblances between some species. For example, the similarity index from leaves of *S. davidii* and *S. mileensis* was 0.93, and the similarity index between leaves of the former and stems of the latter was 0.926. In addition, similarity indexes of stems and leaves from the one species were all above 0.90, which means that the qualities of them were moderately similar. Even so, similarities between them were covered by their vast differences. The result is shown in Table 5.

PCA analysis

The principal component analysis (PCA) analysis of these four plants was employed to show their imparity intuitively. The PCA scatter plot is shown in Figure 5. What we could obtain from the picture is that there is the holistic difference between eight samples. From the figure, the obvious difference existed between stems and leaves of *S. davidii* because they were divided with a far distance at the horizontal negative half axis. Likewise, leaves of *S. angustifolia* and *S. punicea* were distributed closely, which probably implied that they were parallel in certain aspects. However, *S. mileensis* was disparate with other species because its stems and leaves were scattered in the fourth quadrant alone. Furthermore, the fissures between stems and leaves of each species in the scatter plot displayed their differences.

DISCUSSION

Two UV mark peaks of secoiridoids should appear at 240 nm and 270 nm because of the present of additional conjugated double bond.[31] Secoiridoids ingredients, the main principle in our four species, are widespread in the Gentianaceae family,[32] the *Swertia* genus was involved certainly. What is similar with the previous study is that both UV spectra of stems and leaves showed absorption peaks at ca. 240 nm, which implied the existence of abundant secoiridoids in the *Swertia* genus. Nevertheless, it is different with that study that the absorption peaks at 270 nm of *S. davidii*, *S. mileensis*, and *S. punicea* removed to 274 nm nearly. This is probably because of the strong resonating structure consisted of C=O and benzene ring results in red shift in xanthonoids. More than a hundred xanthonoids compounds and its derivatives were also found in members of the genus *Swertia*,[33] which means that

xanthonoids is another big compounds class in this genus.

In the *Swertia*, most secoiridoids and their glycosides derivatives, kinds of big polar ingredient, are distributed widely. Moreover, many positions on benzene rings of most xanthonoids compounds are substituted by hydroxides in this genus. As everyone knows, the more places of benzene rings hydroxides to occupy, the stronger water solubility of xanthonoids is. This is suggested that small polar solutions like petroleum ether were not appropriate to extract compounds in our eight samples.

Swertiamarin, a constituent with the most extensive distribution and the highest content in the genus *Swertia*,[34] possesses significant hepatoprotective properties against D-Galactosamine (D-GaIN)-induced hepatotoxicity.[35] Mangiferin and amarogentin, according to previous researches, possess effects of antioxidant and antidyslipidemic, respectively.[36] As the main constituents to cure liver diseases in *S. mileensis*, swertiamarin is a potential marker to discuss the replacement of the crude drug. Furthermore, the dominant constituent, [Figure 3], is swertiamarin in *S. mileensis* according our experiment. Therefore, it is reasonable that to choose swertiamarin as the standard to find substitutes for *S.mileensis*. In order to evaluate similarity of four species deeply, swertiamarin, sweroside, isoorientin, isovitexin, loganic acid, gentiopicrin, and 6'-*O*-β-D glucopyranosylgentiopicroside were quantified in our experiment for evaluating difference of major active ingredients. Unfortunately, their contents were too low to detect, except for swertiamarin. Therefore, the result of quantification listed swertiamarin only, which suggested the different content of swertiamarin in these four species.

From the UHPLC fingerprint graph of the four species, there are significant differences in type and content of chemical compounds among *S. davidii* and *S. mileensis*. Whether on type or content of chemical compounds, *S. davidii* is richer than *S. mileensis*. The accumulation of metabolites was affected with multiple reasons, such as nature of themselves and environmental factors of light, temperature, soil, and water.[37] Photosynthesis is the trace to gain carbon sources for synthesis of metabolites. Light could control translations of photosynthetic genes, even increase the translation rate.[38] Certainly, the light condition in different altitude is different more or less, so that *S. davidii* collected at lowland (341 ± 5 m above sea level) is significantly incongruent with other three species collected at high elevation (1300–2200 m above sea level) where quantum flux areal density >2000 $\mu mol \bullet m^{-2} \bullet s^{-1}$ occurred more frequently.[39] The variation between *Swertia* species was verified from DNA level by Chassot *et al.*[40] They revealed that nuclear and chloroplast DNA sequence among some *Swertia* species and species was markedly different, even the chloroplast DNA sequence divergence between different species of *Swertia* was higher than between *Swertia* species and other genera. It is interesting that *S. angustifolia* and *S.*

punicea is relatively close compared with others in the PCA analysis, which probably results from one collection place.

Previous studies indicated that all of species we selected show the effect of liver protection. *S. angustifolia* showed significant activities inhibiting the secretion of hepatitis B and e surface antigen.[13] *S. davidii* performed the inhibition of hepatoma carcinoma cell.[41] The total extract of *S. punicea* revealed the function that prevents alaniaetransaminase and aspartate aminotransferase from increasing induced by CCl_4 and BCG / LPS, which is weaker in the latter compared with *S. mileensis*.[42] Through the fingerprint of total constituents, these four species could be evaluated totally to undertake verification of pharmacology if the similarity of constituents exists in theses four species. On the basis of previous researches and our result, although the differences existed among these four species are obvious, *S. davidii* is worth studying further due to its high content of swertiamarin. Swertiamarin possessed potent hepatoprotective properties is the primary constituent to against hepatitis diseases in plants of the genus *Swertia*.[43] In future, *S. davidii* could play a potential role for the resource plant of swertiamarin. Otherwise, many other *Swertia* species and other genus from the family *Gentianaceae* contain swertiamarin, such as *S. japonica*, *Enicostemma axillare*, and *Centaurium erythraea*,[44-46] possibly are new directions to solve problems about substitutes of *S. mileensis* and high price of purchasing drugs in subsequent study.

CONCLUSION

UV and UHPLC methods were developed for the similarity evaluation of four *Swertia* species. Eight samples were analyzed by those two methods, and the chromatographic data were addressed by similarity analysis and PCA. On one hand, results of PCA and similarity analysis proved holistically that a tremendous interspecific distinction exists in these four plants. On the other hand, the quantitative analysis showed the weakly similarity between our samples from local part of swetiamarin, a main bioactive constituent. So, whatever sides we considered, those four *Swertia* members are very different. According to this result, we do not agree with that one of these four species could be considered as the substitute of *S. mileensis*. In order to address issues about substitutes and high cost of purchasing drugs, more studies need to undertake.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. World Health Organization. News release: WHO calls for urgent action to curb hepatitis. Geneva; 2015.

2. World Health Organization. Prevention and control of viral hepatitis infection: framework for global action. 2012;3.

3. Goldstein ST, Zhou F, Hadler SC, Bell BP, Mast EE, Margolis HS. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. Int J Epidemiol 2005;34:1329-39.

4. Editorial Committee of Flora China Nica of the Academy of Sciences of China. Flora of China Beijing Science Press 1998;62:344.

5. State Pharmacopoeia Commission in Chinese Pharmacopoeia. Editorial Board of Chinese Pharmacopoeia. Beijing: Chemistry and Industry Press; 2010. p. 182.

6. Geng CA, Wang LJ, Zhang XM, Ma YB, Huang XY, Luo J, et al. Anti-Hepatitis B Virus Active Lactones from the Traditional Chinese Herb: *Swertia mileensis*. Chem-Eur J 2011;17: 3893-903.

7. Geng CA, Zhang XM, Shen Y, Zuo AX, Liu JF, Ma YB, et al. Swerilactones C and D, anti-HBV new lactones from a traditional Chinese herb: *Swertia mileensis*. Org Lett 2009;11:4838-841.

8. Li XS, Jiang ZY, Wang FS, Ma YB, Zhang XM, Chen JJ. Chemical constituents from herbs of *Swertia mileensis*. China J Chinese Materia Medica 2008;33:2790-793.

9. Kikuzaki H, Kawasaki Y, Kitamura S, Nakatani N. Secoiridoid glucosides from *Swertia mileensis*. Planta Med 1996;62:35-8.

10. Di YT, Liao X, Peng SL, Liang J, Ding LS. A novel monoterpene glycoside from *Swertia mileensis*. Chinese Chem Lett 2003;14:1154-5.

11. Yang YH, Yang FL, Fan J, Zheng L, Yang Y, Zhang WX. Strategy on persistent utilization of *Swertia mileensis*. Chinese J Ethnomed Ethnopharmacy 2003;61:107-9.

12. Zhang XQ, Chen JC, Huang FJ, Tian LY, Tu Y. Anti-HBV activities of xanthones from *Swertia Punicea* Hemsl. North Am J Med Sci 2014;7:73.

13. He K, Ma YB, Cao TW, Wang HL, Jiang FQ, Geng CA, et al. Seven new secoiridoids with anti-hepatitis B virus activity from *Swertia angustifolia*. Planta Med 2012;78:814-20.

14. Tian LY, Bai X, Chen XH, Fang JB, Liu SH, Chen JC. Anti-diabetic effect of methylswertianin and bellidifolin from *Swertia punicea* Hemsl its potential mechanism. Phytomedicine 2010;17:533-9.

15. Bhat GP, Surolia N. *In vitro* antimalarial activity of extracts of three plants used in the traditional medicine of India. Am J Tropical Med Hygiene 2001;65:304-8.

16. Samaddar T, Chaubey B, Jha S, Jha TB. Determination of swertiamarin and amarogentin content and evaluation of antibacterial activity in Eastern Himalayan species of *Swertia* L. Pharmacognosy Communications 2013;3:64-70.

17. Zhang YL. Coefficient of similarity an important parameter infloristic geography. Geographical Res 1998;17:429-34.

18. Chen YJ. Triple connotation of whole view in the traditional Chinese medicine system. Philosophy Res 2009;8:59-64.

19. Tu PF, Shi SP, Jiang Y. Strategies and approaches on exploring material basis of Chinese materia medica. Chinese Traditional Herbal Drugs 2012;43:209-15.

20. Xu GL, Li HL, He JC, Feng EF, Shi PP, Liu YQ, et al. Comparative pharmacokinetics of swertiamarin in rats after oral administration of swertiamarin alone, Qing Ye Dan tablets and co-administration of swertiamarin and oleanolic acid. Journal Ethnopharmacol 2013;149:49-54.

21. Jamwal A. Systematic review on xanthones and others isolates from genus *Swertia*. Int J Pharm Chem Sci 2012;1:1115-33.

22. Geng C A, Zhang X M, Ma Y B, Luo J, Chen JJ. Swerilactones L-O, secoiridoids with C_{12} and C_{13} skeletons from *Swertia mileensis*. J Nat Prod 2011;74:1822-5.

23. Zhu BK, Zhe W, Duan YQ, Wang MF, Gao Y, Wei GZ, et al. Two new xanthones from *Swertia angustifolia*. J Asian Nat Prod Res 2012;14:154-8.

24. Sun YJ, Li XD, Hu FD, Feng SL, Shi GF. Determination of two flavone compounds from *Swertia chirayita* by HPLC. J Lanzhou Med College. 2005;31:57-9.

25. World Health Organization. Guidelines for the assessment of herbal medicines. 1991;1-4.

26. Li YL, Ding CX, Wang HL, Suo YR, You JM, Chen GC. Separation and determination of flavone and xanthone glycosides in Tibetan folk medicinal species *Swertia mussotii* and *S. franchetiana* by capillary electrophoresis. J Anal Chem 2008;63:574-9.

27. Tian W, Chen ZH, Zhai J, Chen LR, Li YM. HPLC fingerprinting of total glycosides of *Swertia franchetiana*. Acta Pharmaceutica Sinica 2005;40:447-52.

28. Gu M, Ouyang F, Su ZG. Comparison of high-speed counter-current chromatography and high-performance liquid chromatography on fingerprinting of Chinese traditional medicine. J Chromatogr A 2004;1022:139-44.

29. Inoue K, Tanada C, Nishikawa H, Matsuda S, Tada A, Ito Y, et al. Evaluation of gardenia yellow using crocetin from alkaline hydrolysis based on ultra high performance liquid chromatography and high-speed countercurrent chromatography. J Sep Sci 2014;37: 3619-24.

30. Jia P. Fingerprints and quality control of traditional Chinese medicine. China Pharmaceuticals 2004;13:79-80.

31. Wolfender JL, Hamburger M, Hostettmann K, Msonthi JD, Mavi S. Search for bitter principles in *Chironia* species by LC-MS and isolation of a new secoiridoid diglycoside from *Chironia krebsii*. J Nat Prod 1993;56:682-9.

32. Singh A. Phytochemicals of Gentianaceae: a review of pharmacological properties. Int J Pharmaceutical Sci Nanotechnol 2008;1:33-6.

33. Brahmachari G, Mondal S, Gangopadhyay A, Gorai D, Mukhopadhyay B, Saha S. *Swertia* (Gentianaceae): chemical and pharmacological aspects. Chem Biodiver 2004;1:1627-51.

34. Liu T, Liu Y, Li T. Medicinal ethnic botany research of *Swertia* genus. Nat Prod Res Dev 2015;27:191-7.

35. Jaishree V, Badami S. Antioxidant and hepatoprotective effect of swertiamarin from *Enicostemma axillare* against D-galactosamine induced acute liver damage in rats. J Ethnopharmacol 2010;130:103-6.

36. Asthana RK, Gupta R, Agrawal N, Srivastava A, Chaturvedi U, Kanojiya S, *et al*. Evaluation of antidyslipdemic effect of mangiferin and amarogentin from *swertia chirayita* extract in HFD induced charles foster rat model and *in vitro* antioxidant activity and their docking studies. Int J Pharmaceutical Sci Res 2014;5:3734.

37. Eller CB, Burgess SSO, Oliveira RS. Environmental controls in the water use patterns of a tropical cloud forest tree species, *Drimys brasiliensis* (Winteraceae). Tree Physiol 2015;35:387-99.

38. Wu SH. Gene expression regulation in photomorphogenesis from the perspective of the central dogma. Annu Rev Plant Biol 2014;65:311-33.

39. Korner C, Diemer M. In situ photosynthetic responses to light, temperature and carbon dioxide in herbaceous plants from low and high altitude. Funct Ecol 1987:179-94.

40. Chassot P, Nemomissa S, Yuan Y M, Küpfer P. High paraphyly of *Swertia* L.(Gentianaceae) in the Gentianella-lineage as revealed by nuclear and chloroplast DNA sequence variation. Plant Syst Evol 2001;229:1-21.

41. Zhao LJ, Zuo ZC, Zou HB, Liu XM. Distilling swertiamarin from *Swertia davidi* Franch and its effect on resisting cancer cells. Guiding J TCM 2006;12:62-4.

42. Peng F, Liu XB, Fang CS, Yang ZK, Zhang HM. Protective effect of *Swertia punicea* on experimental hepatic injury. Traditional Chinese Drug Res Clin Pharmacol 2002;13:376-8.

43. Jaishree V, Badami S. Antioxidant and hepatoprotective effect of swertiamarin from *Enicostemma axillare* against D-galactosamine induced acute liver damage in rats. J Ethnopharmacol 2010;130:103-6.

44. Yamahara J, Kobayashi M, Matsuda H, Aoki S. Anticholinergic action of *Swertia japonica* and an active constituent. J Ethnopharmacol 1991;33:31-5.

45. Jaishree V, Badami S, Kumar MR, Tamizhmani T. Antinociceptive activity of swertiamarin isolated from *Enicostemma axillare*. Phytomedicine 1991;16:227-32.

46. Kumarasamy Y, Nahar L, Cox PJ, Jaspars M, Sarker SD. Bioactivity of secoiridoid glycosides from *Centaurium erythraea*.. Phytomedicine 2003;10:344-7.