Establishment of a Method for The Distinguishment of Taxilli Herba Parasitizing Mulberry, Willow, and Cinnamon Based on Dual-Component Measurement

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Abstract

Background: Taxilli Herba (TH) is a commonly used Chinese medicinal herb that parasitizes different tree types, but objective methods to determine its quality and detect contamination from host substances have not been reported. Thus, we aimed to establish methods to improve the safety and quality of TH.

Methods: We collected 10 samples each of TH hosted by mulberry, willow, and cinnamon trees from different regions and batches, along with twigs of the corresponding host trees. Samples were prepared by ultrasonic extraction with methanol as a solvent. HPLC was used to measure the content of quercitrin, the intrinsic component of TH, as well as of morusin, salicin, and cinnamaldehyde, characteristic of mulberry, willow, and cinnamon, respectively.

Results: Quercitrin was detected in all host trees but not in host twigs. However, the accumulation of the characteristic components of the hosts in TH substantially varied among the three hosts. The contents of morusin, salicin, and cinnamaldehyde in mulberry, willow, and cinnamon twigs were 134.78-437.60, 1787.91-2564.65 and 7219.36-10783.21 µg/g respectively, and the corresponding values in TH obtained from mulberry, willow, and cinnamon were 0.27-4.27, 102.62-545.83 and 11.33-120.97 µg/g, respectively.

Conclusions: The host influences TH quality to varying degrees via the transfer of characteristic components. In this study, we successfully detailed a potentially generalizable, accurate, and reproducible dual-component measurement method to identify the TH host.

Background

Taxilli Herba (TH) is a commonly used herb in traditional Chinese medicine. As stipulated by the Pharmacopoeia of the People's Republic of China (Chinese Pharmacopoeia), TH comprises the dried stems and branches of *Taxillus chinensis* (DC.) Danser [1]. Also known as mulberry mistletoe, *T. chinensis* is a hemi-parasitic herb that parasitizes mulberry trees and many other tree species, such as peach, plum, longan, lychee, starfruit, camellia, tung, rubber, Chinese banyan, cotton, horsetail pine, and water pine [2]. In addition to stipulating the origin of TH, the Chinese Pharmacopoeia requires that it should not contain detectable cardiac glycosides; the pharmacopoeia does not impose other restrictions on the host plants. In other words, with the exception of cardiac glycoside-containing plants, various plant species can serve as sources of TH. Materia Medica textual research is not difficult to find that all the records of Materia Medica in the past are named after the host, that is, the Taxilli Herba of the host is the mulberry tree. Other hosts outside the mulberry tree such as poplar, maple, willow, peach, mist, and beech are also named after the host. Compendium of Materia Medica includes parasitism of mulberry trees, parasitism of peach trees and willow parasitism. Later, some experts conducted a lot of investigations on the parasitic medicinal materials from different host sources, and found that the hosts were different, and the functions and indications were different. For example, the parasitic medicinal materials of the Daluo umbrella host were used to treat bone injuries, and the parasitic medicinal materials of the mountain sesame host were used to treat colds, for tonsillitis, etc., different parasitic herbs have different effects.

Our previous research found that it is impossible to distinguish different parasitic medicinal materials in terms of traits and microscopy. Therefore, it is important to establish a method to distinguish different host parasitic medicinal materials.

Several studies have demonstrated that host plants not only provide water and inorganic salts to TH but also transfer their characteristic secondary metabolites. These can accumulate in TH and could affect its quality to varying degrees [3–8]. Much research has been carried out to study the host influence on the components and pharmacological effects of *Viscum coloratum* or *Viscum album* found that the phenolic compounds of mistletoe from different hosts are different, and the types of compounds also differ. For example, mistletoe that parasitizes *Robinia pseudoacacia* contains gallic acid, and that of mistletoe is contained in hosts such as ash trees. The aforementioned mistletoe does not have this ingredient [9].

Further, the transfer of toxic components of the host to the mulberry parasite might confer host toxicity to the mulberry parasite, which will affect the quality and safety of the medicinal materials to varying degrees [10–11]. However, due to the different hosts, the pharmacological effects and efficacy of the mulberry parasites are not the same, and this might affect the stability, effectiveness, and quality of the medicinal materials to varying degrees [12–13]. In the past, TH components have been named after their host plants to prevent the inadvertent harvesting and use of undesirable parts of TH [14]. However, the results of recent research have indicated that TH obtained from different host plants cannot be distinguished by differences in traits or microscopic characteristics [15].

To the best of our knowledge, studies on the identification of host plants of TH or methods for its quality control have not been reported. In the present study, TH samples harvested from mulberry, willow, and cinnamon trees were used as experimental materials. The contents of quercitrin, an inherent component of TH, as well as morusin, salicin, and cinnamaldehyde, which are the respective characteristic components of mulberry, willow, and cinnamon trees, were measured using a dual-component measurement method, evaluating the inherent component of TH and the characteristic component of each host plant. By investigating the transfer and accumulation of characteristic host plant components in TH, we aimed to develop a method to determine the possible influences of host plants on TH quality and identify the host plants, in addition to exploring effective methods for the quality control of TH medicinal materials from multiple host sources.

Methods

Methods and materials

Plant material

TH was harvested from the wild in Guangxi Province, China; the source plants were identified as *T. chinensis* by Professor Wei Songji, a Research Fellow at the College of Pharmacy, Guangxi University of Chinese Medicine. The host species of the collected TH samples were *Morus alba* L. (mulberry), *Salix*

babylonica L. (willow), and *Cinnamomum cassia* Presl (Chinese cinnamon). Information related to sample collection is presented in Tables 1–3.

Table 1
Location and date of collection for Taxilli Herba parasitizing mulberry samples.

Sample number	Collection location	Collection date (year. month)
1	Cangwu county, Wuzhou city	2019.01
2	Jinxiu county, Laibin city	2019.02
3	Fuchuan county, Hezhou city	2019.02
4	Debao county, Baise city	2019.01
5	Xingye county, Yulin city	2019.01
6	Cenxi city, Wuzhou	2019.02
7	Rong county, Yulin city	2019.01
8	Qinbei District, Qinzhou city	2019.02
9	Qinnan District, Qinzhou city	2019.02
10	Qingxiu District, Nanning city	2019.02
Note: For each sample, both Taxilli Herba (THM) and host twig (MB) specimen were collected.		

Table 2
Location and date of collection for Taxilli Herba parasitizing willow samples.

Sample number	Collection location	Collection date (year.month)
1	Fangcheng district, Fangchenggang city	2019.01
2	Lingshan county, Qinzhou city	2019.01
3	Shangsi county, Fangchenggang city	2019.02
4	Cenxi city, Wuzhou	2019.02
5	Rong county, Yulin city	2019.01
6	Shanglin county, Nanning city	2019.02
7	Qinbei district, Qinzhou city	2019.02
8	Qingxiu district, Nanning city	2019.02
9	Jingxi city, Baise	2019.01
10	Wuming county, Nanning city	2019.02
Note: For each sample, both Taxilli Herba (THW) and host twig (WB) specimen were collected.		

Table 3
Location and date of collection for Taxilli Herba parasitizing cinnamon samples.

Sample number	Collection location	Collection date (year.month)
1	Dongxing city, Fangchenggang city	2019.01
2	Ningming county, Chongzuo city	2019.01
3	Lingshan county, Qinzhou city	2019.01
4	Shangsi county, Fangchenggang city	2019.02
5	Teng county, Wuzhou city	2019.02
6	Cangwu county, Wuzhou city	2019.02
7	Rong county, Yulin city	2019.02
8	Cenxi city, Wuzhou	2019.02
9	Pingnan county, Guigang city	2019.02
10	Fangcheng district, Fangchenggang city	2019.02
Note: For each sample, both Taxilli Herba (THC) and host twig (CB) specimen were collected.		

For the TH samples, floriferous branches of *T. chinensis* from different host trees were treated in accordance with the procedure described in the Chinese Pharmacopoeia. For host samples, twigs were collected from the mulberry, willow, and cinnamon trees from which TH was harvested. Each sample was dried in the shade, placed in an oven at 45 °C for 3 h, ground, and then sieved through a four-mesh screen to obtain respective TH and host tree sample powders.

Measurement of quercitrin content in TH samples

Preparation of quercitrin reference solution

Quercitrin (6.34 mg) was accurately weighed and placed in a 10-mL volumetric flask. The volume of the sample was made up with methanol, and the contents of the flask were uniformly mixed by shaking to obtain a reference solution with a quercitrin concentration of 634.00 mg/L.

Preparation of sample solutions

TH sample powder (0.50 g) was accurately weighed and placed in a 100-mL conical flask (with a stopper). To this, 25 mL of 75% methanol was added; the resulting mixture was weighed, subjected to ultrasonic vibration for 45 min, and cooled. Then, the volume was made up to compensate for mass loss and passed through a 0.45-μm membrane filter to obtain the sample solution.

HPLC conditions and system suitability

Full-wavelength scanning (within the wavelength range of 190–400 nm) was performed using the Waters photodiode array (PDA) detector. The maximum absorption for quercitrin was obtained at 256 nm; therefore, 256 nm was set as the detection wavelength for quercitrin. HPLC was performed using the Waters C₁₈ column (5 µm, 4.6 mm × 250 mm) at a flow rate of 1.0 mL/min, a column temperature of 30 °C, an injection volume of 10 µL, and a run time of 24 min. The mobile phase was acetonitrile (A) and 0.1% phosphoric acid (B), with an elution gradient for 0–24 min with 18% A–32% A and 82% B–68% B.

Measurement of the contents of characteristic components of the host in TH samples

Preparation of reference solutions

Morusin (3.36 mg), salicin (6.24 mg), and cinnamaldehyde (4.80 mg) were precisely weighed and added to 10-, 25-, and 50-mL volumetric flasks, respectively. The volume of the sample in each flask was made up with methanol, and the flask contents were uniformly mixed by shaking to obtain reference solutions with concentrations of 336.00, 249.60, and 96.0 µg/mL, respectively.

Preparation of sample solutions

Preparation of sample solutions were referenced to the optimization method of Benwei Su [16]. Approximately 0.50 g of TH sample powder was obtained from each host and added to separate 10-mL conical flasks (with stoppers). Twenty-five milliliters of 75% methanol were added to the flask, and the resulting mixture was weighed, subjected to ultrasonic vibration for 45 min, cooled, topped up to compensate for mass loss, and passed through a 0.45-µm membrane filter to obtain the sample solution.

Determination of detection wavelengths

Full-wavelength scanning (within the wavelength range of 190–400 nm) was performed using the Waters PDA detector, and the maximum absorption of morusin, salicin, and cinnamaldehyde was at 269, 211, and 290 nm, respectively. These were therefore set as the detection wavelengths for these components.

HPLC conditions for the detection of morusin, salicin, and cinnamaldehyde

HPLC was carried out using the Waters C₁₈ column (5 µm, 4.6 mm × 250 mm) with a volumetric flow rate of 1.0 mL/min, a column temperature of 30 °C, and an injection volume of 10 µL. The mobile phase for morusin, salicin, and cinnamaldehyde comprised acetonitrile (A) and 0.1% phosphoric acid (B). The gradient elution procedure of morusin, salicin, and cinnamaldehyde are presented in Table 4.

Table 4
Gradient elution procedure for morusin, salicin, and cinnamaldehyde

Ingredient	T (min)	acetonitrile (A, %)	0.1% phosphoric acid (B, %).
morusin	0	18	82
	45	72	18
	55	72	28
salicin	0	5	95
	15	15	85
	45	32	68
cinnamaldehyde	0	18	82
	25	32	68
	32	40	60

Results

Determination of quercitrin, the inherent component of TH

Ten microliters each of the quercitrin reference and sample solutions was precisely drawn and injected into the HPLC system under the conditions specified previously herein, and the chromatogram was recorded (Fig. 1). The HPLC results showed that the baseline separation of quercitrin with symmetric peaks and a high degree of separation could be achieved, indicating that the adopted test method had high specificity.

Investigation of the linear relationship

The quercitrin reference solution was diluted with methanol to obtain working solutions with concentrations of 6.34, 12.68, 25.36, 126.80, 317.00, and 634.00 mg/L. Ten microliters of each working solution was injected into the HPLC system for the measurement of peak areas, and regression analysis was performed by plotting the peak area against the quercitrin concentration. The regression equation obtained was $Y = 2.64 \times 10^7 X + 4947.87$, with $R^2 = 0.9999$ within the concentration range of 6.34–634.00 mg/L, which indicates a good linear relationship between the quercitrin concentration and peak area.

Precision test

The quercitrin reference solution was precisely drawn, and six consecutive samples were injected into the HPLC system. The relative standard deviation (RSD) of the calculated peak areas was 0.18%, which fulfilled the requirements for quantitative analysis stipulated by the Chinese Pharmacopoeia.

Stability test

Powdered samples (0.5 g) of Taxilli Herba parasitizing mulberry (THM) were precisely weighed and used to prepare sample solutions. Quercitrin content in the sample solutions was measured by HPLC at 5-h intervals for a total duration of 24 h. The calculated RSD was 0.89%, which indicates that the quercitrin content in the sample solutions remained stable within 24 h.

Reproducibility test

Six portions of THM, each weighing 0.50 g, were precisely weighed and used to prepare TH sample solutions. Quercitrin content in the sample solutions was measured by HPLC under the conditions described in Sect. 2.4.3. The calculated RSD was 0.64%, which indicates that the adopted test method provided good reproducibility.

Sample recovery test

Six portions of THM-1 powder with known quercitrin content (2.29 mg/g) were precisely weighed, and then 567.00 µg of the quercitrin reference was precisely measured and added to each portion. Sample solutions were prepared and injected into the HPLC system to calculate the quercitrin content from the peak area. The results indicated an average quercitrin recovery rate of 99.51% and an RSD value of 2.86% (Table 5), demonstrating the reproducibility of the test method.

Table 5
Test results of quercitrin addition and recovery in Sample No. 1 (n = 6).

No.	Original amount (µg)	Additional amount (µg)	Measured quantity (µg)	Recovery rate (%)	Average recovery rate(%)	RSD (%)
1	567.28	567.00	1161.88	104.87	99.51	2.86
2	568.79	567.00	1121.88	97.55		
3	578.10	567.00	1143.69	99.75		
4	571.83	567.00	1137.55	99.77		
5	570.66	567.00	1122.41	97.31		
6	575.41	567.00	1129.90	97.79		

Measurement of quercitrin content

The powdered TH samples and powdered host tree samples were used to prepare the sample solutions The quercitrin content in various sample solutions was measured by HPLC. As shown in Table 6, TH hosted by mulberry, willow, and cinnamon all contained quercitrin, but the twigs of all hosts did not. Therefore, it could be deduced that quercitrin is an inherent component of the TH samples and that the quercitrin content of TH hosted by the different trees might affect the quality of TH.

Table 6

Contents of quercitrin in Taxilli Herba and its host plant. Items indicated by different lowercases in each column are significantly different (determined by Tukey HSD test, $p < 0.05$, means $\pm$ SD, $n = 10$)

Sample name	Content (mg/g)										mean $\pm$ SD
	1	2	3	4	5	6	7	8	9	10	
THM	2.29	1.98	2.10	2.16	2.64	2.77	2.10	2.82	2.33	3.11	2.43 $\pm$ 0.38 ^{ab}
THW	3.00	3.00	2.81	2.36	2.15	2.47	5.03	2.45	2.94	2.91	2.91 $\pm$ 0.80 ^a
THC	2.25	1.94	2.33	1.82	1.46	1.79	1.95	2.92	1.89	2.25	2.06 $\pm$ 0.40 ^b
MB	-	-	-	-	-	-	-	-	-	-	-
WB	-	-	-	-	-	-	-	-	-	-	-
CB	-	-	-	-	-	-	-	-	-	-	-
Note: "-" means not detected.											

Measurement of characteristic component contents in TH host samples

Ten microliters each of morusin, salicin, or cinnamaldehyde reference solutions and the sample solutions of TH hosted by mulberry, willow, and cinnamon was precisely drawn and injected into the HPLC system. The chromatograms were then recorded (Figs. 2–4). The HPLC results showed that the baseline separation of morusin, salicin, and cinnamaldehyde could be achieved, with symmetric peaks and a high degree of separation, indicating that the adopted method had high specificity.

Investigation of linear relationships

Morusin, salicin, and cinnamaldehyde reference solutions were diluted with methanol to obtain working solutions of the following concentrations: 0.10, 0.25, 0.42, 0.84, 1.68, and 3.36 $\mu\text{g/mL}$ (morusin); 0.12, 0.24, 0.62, 1.24, 3.12, and 6.24 $\mu\text{g/mL}$ (salicin); 0.96, 1.92, 3.84, 5.76, 7.68, and 9.60 $\mu\text{g/mL}$ (cinnamaldehyde). Ten microliters of each working solution was injected into the HPLC system to measure the peak area, and regression analysis was performed by plotting peak area against concentration. Table 7 shows the obtained regression equations and linear ranges of morusin, salicin, and cinnamaldehyde.

Table 7
Regression equations and linear ranges of morusin, salicin and cinnamaldehyde

Components	Regression equation	R ²	Linearity (µg/mL)
morusin	$y = 3.01 \times 10^6 x - 112.85$	0.9999	0.10 ~ 3.36
salicin	$y = 1.63 \times 10^4 x + 8.64$	0.9999	0.12 ~ 6.24
cinnamaldehyde	$y = 9.62 \times 10^7 x - 2765.73$	0.9999	0.96 ~ 9.60

Precision test

Morusin, salicin, and cinnamaldehyde reference solutions were used for five consecutive injections of 10 µL each into the HPLC system to measure the peak areas and calculate the content of each component. The respective RSD values of morusin, salicin, and cinnamaldehyde were 0.85%, 2.83%, and 0.11%; these are indicative of high instrument precision.

Stability test

The contents of the sample solutions were tested at 0, 1, 2, 4, 6, 12, and 24 h. The respective RSD values of morusin, salicin, and cinnamaldehyde were 1.75%, 2.71%, and 2.51%, which indicate that the samples remained stable within 24 h.

Reproducibility test

Six portions of each TH sample obtained from mulberry, willow, and cinnamon trees were precisely weighed and used to prepare sample solutions. The solutions (10 µL) were then injected into the HPLC system to measure peak areas and calculate the content of each component. The average content and RSD values were 3.06 µg/g and 2.86% for morusin, 522.26 µg/g and 2.72% for salicin, and 117.13 µg/g and 2.44% for cinnamaldehyde, respectively, indicating that the adopted method presented good reproducibility.

Sample recovery test

Six portions of each TH sample of known concentrations (3.06 µg/g morusin, 522.26 µg/g salicin, and 117.13 µg/g cinnamaldehyde) were precisely weighed. Morusin (0.77 µg), salicin (130.00 µg), and cinnamaldehyde (20.00 µg) were used to prepare sample solutions. Each solution was injected into the HPLC system using an injection volume of 10 µL. The results indicated that morusin, salicin, and cinnamaldehyde had a recovery rate of 94.84%, 95.72%, and 97.85% and RSD value of 2.17%, 1.28%, and 2.37%, respectively, as shown in Table 8.

Table 8
Recovery test of morusin, salicin, and cinnamaldehyde

Sample	Original amount ($\mu\text{g/g}$)	Additional amount ($\mu\text{g/g}$)	Measured quantity ($\mu\text{g/g}$)	Recovery rate (%)	Average recovery rate(%)	RSD (%)
morusin	0.77	0.77	1.49	93.59	94.84	2.17
	0.78	0.77	1.51	96.21		
	0.75	0.77	1.48	96.08		
	0.73	0.77	1.43	91.24		
	0.79	0.77	1.52	96.60		
	0.78	0.77	1.51	95.29		
salicin	29.16	20.00	48.04	94.41	95.72	1.28
	28.66	20.00	47.95	96.46		
	29.78	20.00	49.04	96.33		
	30.23	20.00	49.26	95.15		
	29.56	20.00	49.05	97.47		
	28.32	20.00	47.22	94.51		
Cinnamaldehyde	130.00	130.00	256.12	97.02	97.85	2.37
	128.13	130.00	254.21	96.99		
	132.14	130.00	262.22	100.06		
	135.45	130.00	261.01	96.59		
	125.36	130.00	257.02	101.28		
	132.32	130.00	256.02	95.15		

Measurement of morusin, salicin, and cinnamaldehyde content

Each sample (0.5 g) was precisely weighed and used to prepare sample solutions. The sample solutions were injected into the HPLC system to measure the content of each component. The result is shown in Figs. 4–6, indicated the presence of morusin and the absence of salicin and cinnamaldehyde in TH hosted by mulberry, the presence of salicin and the absence of morusin and cinnamaldehyde in TH hosted by willow, and the presence of cinnamaldehyde and the absence of morusin and salicin in TH hosted by cinnamon. These results confirm that morusin, salicin, and cinnamaldehyde are the respective characteristic components of mulberry, willow, and cinnamon and that morusin, salicin, and

cinnamaldehyde components in TH were transferred from the corresponding host trees. The contents of morusin, salicin, and cinnamaldehyde in mulberry, willow, and cinnamon twigs were 134.78–437.60, 1787.91–2564.65 and 7219.36–10783.21 µg/g respectively, and the corresponding values in TH obtained from mulberry, willow, and cinnamon were 0.27–4.27, 102.62–545.83 and 11.33–120.97 µg/g, respectively.

Discussion

Wild-growing TH exhibits complex and diverse biological characteristics. The Grand Dictionary of Chinese Traditional Medicine states that more than 50 plant species belonging to 29 families can serve as hosts for TH [17]. The results of a survey conducted by Zhu et al. on the sources of TH within Guangxi Province also indicated that more than 150 plant species belonging to 36 families within the province could serve as its hosts [18]. In recent years, studies on the chemical components, toxicity, and pharmacological effectiveness of TH have confirmed that the host characteristics are the key factors influencing quality [19]. Different hosts lead to different chemical composition, toxicity, and pharmacological performance of parasitic medicinal materials. Therefore, identifying the source of different hosts is the key to quality control of medicinal materials.

In the Chinese Pharmacopoeia, quercetin serves as the key indicator of TH quality. However, previous studies have indicated that quercetin exists mostly as quercitrin in TH, resulting in a low free quercetin content [20, 21]. Therefore, the quantitative detection of quercetin does not provide a true reflection of TH quality. In the present study, the quercitrin content was measured in TH hosted by mulberry, willow, and cinnamon trees, as well as in the corresponding hosts. Quercitrin was not detected in twigs from the three trees but was detected in TH hosted by the trees. Our results indicated that quercitrin is an inherent and specific component of TH and does not typically differ significantly among TH grown on different hosts. The transfer relationship between the TH and the host provides a new research direction. Although the content of morusin obtained from the TH-parasitized mulberry branch was low, we believe this was an exceptional result and not indicative of a trend. Other substances that might be more freely transferred between the host and parasite in mulberry must be investigated.

In the present study, the morusin, salicin, and cinnamaldehyde contents in TH hosted by mulberry, willow, and cinnamon trees were measured. The results indicated that the host trees were capable of transferring their characteristic components to TH, which could be detected through HPLC. Therefore, this method can effectively identify TH hosts. The fully parasitic plant *Cuscuta chinensis* can communicate with host plants (via materials such as DNA, RNA, protein, and metabolites), and the genomic DNA of the host plant can be transferred (HGT) to the parasitic plant *C. chinensis* [22–24]. Further, the semi-parasitic plant Han mistletoe (*Viscum album* var. *Coloratum*) has different compositions of carbohydrates, crude protein, crude fiber, and minerals with different hosts [25].

In the Chinese Pharmacopoeia, the non-detection of cardiac glycosides in TH is used as the criterion to ascertain the absence of toxic influences of toxin-containing hosts on TH. However, a study on TH toxicity by Zhou et al. revealed that in addition to TH obtained from cardiac glycoside-containing

oleander plants, TH parasitizing non-cardiac glycoside-containing cashew trees was toxic [9]. It is therefore evident that the quality control criterion for TH described in the Chinese Pharmacopoeia no longer provides complete assurance of its safety. Consequently, effective identification of the hosts of TH plays a critical role in the establishment of methods for its quality control.

Quercitrin, morusin, salicin, and cinnamaldehyde possess known biological activities [26–29]. The latter three substances are transferred from their respective host trees and accumulate within TH. Through detection of the mulberry parasitic medicinal materials morusin, salicin, and cinnamaldehyde, the host source of the mulberry, willow, and cinnamon host TH medicinal materials can be identified.

Conclusions

For TH parasitizing various host trees, the host influences the its quality to varying degrees via the transfer of characteristic components. The establishment of quality control methods based on dual-component measurements can thus provide an accurate, convenient, and reproducible scientific basis for host identification when TH is obtained from a diverse range of host plants. This can be used to identify the host source of TH medicinal materials and establish a control method based on the detection of "dual-substance components", which provides a theoretical basis for the quality control of TH medicinal materials from complex hosts. At the same time, it also provides a reference method for future research on other host sources, especially toxic hosts, such as oleander, sumac, and neem, among others.

Abbreviations

TH, Taxilli Herba; THM, Taxilli Herba parasitizing mulberry; THW, Taxilli Herba parasitizing willow; THC, Taxilli Herba parasitizing cinnamon; MB, mulberry branch; WB, willow branch; CB, cinnamon branch.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing interests

The authors have no conflict of interest to declare.

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Authors' contributions

CZS, RM, QWH and LYH designed the experiments. CZS, LLZ, and LRY performed the research. CZS and RM analyzed the data and drafted the manuscript. SBW and LHL revised the manuscript and contributed reagents/materials/analysis tools. All authors read and approved the final manuscript.

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References

1. Pharmacopoeia Committee of China. Chinese Pharmacopoeia. Beijing: Chemical Industry Publishing House; 2015. p. 299. (in Chinese).
2. China Flora Editorial Board. vol. 24, Flora China, 1988, pp. 131. (in Chinese).
3. Hu KF, Li YH, Du YK, Su BW, Lu D. Analysis of 1-deoxynojirimycin component Correlation between medicinal parasitic loranthus from loranthaceae and their mulberry host trees. Journal of Medicinal Plants Research. 2011;5:4326–31.
4. Lu D, Su BW, Li YH, Zhu KX, Pei HH, Zhao FH, Li J. Study on salicin content correlation between taxilli herba and their willow host plants. Journal of Medicinal Plant Research. 2012;6:2474–7.
5. Zhang XJ, Su BW, Li J, Li YH, Lu D, Zhu KX, Pei HH, Zhao MH. Analysis by RP-HPLC of mangiferin component correlation between medicinal loranthus and their mango host trees. J Chromatogr Sci. 2014;52:1–4.
6. Liu RY, Su BW, Huang FY, Ru M, Zhang H, Qin ZM, Li YH, Zhu KX. Identification and analysis of cardiac glycosides in Loranthaceae parasites Taxillus chinensis (DC.) Danser and Scurrula parasitica Linn. and their host Nerium indicum Mill. J Pharm Biomed Anal. 2019;174:450–9.
7. Su BW, Li YH, Lu D, Zhu KX, Pei HH, Zhao MH, Li J. Determination of berberine in Taxilli Herba and its host plants -Clausena lansium by RP-HPLC. World Science and Technology (Modernization of Traditional Chinese Medicine and Materia Medica). 2012; 14:1891–1894. (in Chinese with English abstract).
8. Li YH, Su BW, Zhang XJ, Zhu KX, Pei HH, Zhao MH, Lu D. Influence of Host Plant on the Volatile Components of Taxilli Herba. Lishizhen Medicine Materia Medica Research. 2012;23:574–8. (in

- Chinese with English abstract).
9. Vicas SI, Rugina D, Leopold L, et al. HPLC fingerprint of bioactive compounds and antioxidant activities of *Viscum album* from different host trees. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. 2011;39:48–57.
 10. Zhou HH, Liu XL, Qian HB, Wang SQ, Zhou C. Comparative Study on Toxicities of *Taxillus sutchuenensis* from Different Host Plants. *Chinese Journal of Experimental Traditional Medical Formulae*. 2013;19:274–7. (in Chinese with English abstract).
 11. Chen JY, Zhou F. Comparative Study on Toxicities of *Taxillus sutchuenensis* from Different Host Plants. *Lishizhen Medicine Materia Medica Research*. 2008;19:2418–9. (in Chinese with English abstract).
 12. Zhang H, Huang YY, Liu RY, Lu HL, Su BW, Zhu KX, Li YH. Effects of *Taxilli Herba* from different hosts on lowering blood pressure of spontaneously hypertensive rats. *Chinese Traditional Patent Medicine*. 2018;40:249–54. (in Chinese with English abstract).
 13. Zhou BJ, Chen BH, Wang JJ, Lai JJ, Xiao YJ. Study on Hydroxyl Radical Scavenging Activities of Leaves Extracts of *Scurrula parasitica* Sourced from Five Different Host Trees. *Journal of Fujian Normal University (Natural Science Edition)*. 2010;26:84–9. (in Chinese with English abstract).
 14. L M, Z S. *Compendium of Materia Medica*, Vol. 2. People's Medical Publishing House, 1982, pp. 2158 (in Chinese).
 15. Li YJH, Lu L, Qiao X, Yu L, Li YH. Microscopic identification characteristics of *Taxilli Herba* from two different hosts. *Journal of Chinese Medicinal Materials*. 2016;39:1007–9. (in Chinese with English abstract).
 16. Su BW, Zhang XJ, Zhu KX, Zhao MH, Pei HH, Li J, Li YH. Optimization of Extraction Technology of Quercitrin from *Taxillus chinensis*. 2013; 19:8–10. (*Chinese Journal of Experimental Traditional Medical Formulae*).
 17. Jiangsu New Medical College. *Dictionary of Traditional Chinese Medicine*. Shanghai: Shanghai Science and Technology Publishers; 1986. (in Chinese).
 18. Zhu KX, Lu D, Pei HH, Zhao MH, Li YH. Investigation on the Distribution and Host Status of *Taxilli Herba* in Guangxi. *Guangxi Journal of Traditional Chinese Medicine*. 2010;33:59–61. (in Chinese with English abstract).
 19. Zhang H, Huang YY, Su BW, Zhu KX, Lu HL, Yin SG, Guo M, Li YH. Effect of host on quality of *Taxilli Herba*. *Modernization of Traditional Chinese Medicine and Materia Medica-World. Science Technology*. 2016;18:1182–7. (in Chinese with English abstract).
 20. Zhang XJ, Zhu KX, Zhao MH, Pei HH, Li YH. Content Analysis of Quercitrin and Quercetin in *Taxilli Herba* from Different Host Plant. *Lishizhen Medicine Materia Medica Research*. 2011;22:1604–6. (in Chinese with English abstract).
 21. Su BW, Zhang XJ, Zhu KX, Zhao MH, Pei HH, Li J, Li YH. Comparison of methods for the determination of Quercitrin and Quercetin in *Taxilli Herba* by RP-HPLC. *Guangxi Journal of Traditional Chinese Medicine*. 2012;35:53–5. (in Chinese with English abstract).

22. Saima SGK, Nathan R, Johnson, Eric W, Feng W, Ceyda C, Vivian B, Tamia P, Claude W., James H, Westwood MJ. Axtell. MicroRNAs from the parasitic plant *Cuscuta campestris* target host messenger RNAs. *Nature: International weekly journal of science*. 2018;553:82–5.
23. Sun G, Xu Y, Liu H, Sun T, Zhang JX, Hettenhausen, Christian, Shen GJ, Qi JF, Qin Y, Li J, Wang L, Chang W, Guo ZH, Baldwin IT, Wu JQ. Large-scale gene losses underlie the genome evolution of parasitic plant *Cuscuta australis*. *Nature communications*. 2018;9:2683.
24. Vogel ASR, Denton AK, Usadel B, Hollmann J, Fischer K, Bolger A, Schmidt MHW, Bolger ME, Gundlach H, Mayer FX. Weiss-Schneeweiss Hanna, Temsch EM, Krause Kirsten. Footprints of parasitism in the genome of the parasitic flowering plant *Cuscuta campestris*. *Nature communications*. 2018;9:2683.
25. Chul WK, Chan HA, Hyun S, Lee J, Yi EJC, Sang HL, Hee YK. Proximate and mineral components of *Viscum album* var. *coloratum* grown on eight different host tree species. *Journal of Forestry Research*. 2019;30:1245–53.
26. Yang L. Research Development of Pharmacology Activities of Quercitrin. *Asia-Pacific Traditional Medicine*. 2015;11:61–3. (in Chinese with English abstract).
27. Pang DR, Liao ST, Wang WF, Mu LX, Li E, Shen WZ, Liu F, Zou YX. Destruction of the cell membrane and inhibition of cell phosphatidic acid biosynthesis in *Staphylococcus aureus*: an explanation for the antibacterial mechanism of morusin. *Food Funct*. 2019;10:6438–46.
28. Mohd S, Sidney JS. Efficacy and Safety of White Willow Bark (*Salix alba*) Extracts. *Phytother Res*. 2015;29:1112–6.
29. Song ZH, Zhang YW, Wang LJ, Yu BT. Pharmacological Activity and Research Progress of Cinnamaldehyde. *Pharmaceutical Journal of Chinese People's Liberation Army*. 2018;34:550–4. (in Chinese with English abstract).

Figures

Figure 1

HPLC chromatogram of quercitrin in reference solutions and sample solutions.

Figure 2

HPLC chromatogram of morusin in reference solutions and sample solutions. THM, Taxilli Herba parasitizing mulberry; MB, mulberry branch; THW, Taxilli Herba parasitizing willow; WB, willow branch; THC, Taxilli Herba parasitizing cinnamon; CB, cinnamon branch.

Figure 3

HPLC chromatogram of salicin in reference solutions and sample solutions. THM, Taxilli Herba parasitizing mulberry; MB, mulberry branch; THW, Taxilli Herba parasitizing willow; WB, willow branch; THC, Taxilli Herba parasitizing cinnamon; CB, cinnamon branch.

Figure 4

HPLC chromatogram of cinnamaldehyde in reference solutions and sample solutions. THM, Taxilli Herba parasitizing mulberry; MB, mulberry branch; THW, Taxilli Herba parasitizing willow; WB, willow branch; THC, Taxilli Herba parasitizing cinnamon; CB, cinnamon branch.

Figure 5

Contents of morusin in 10 batches of Taxilli Herba and its host. THM, Taxilli Herba parasitizing mulberry; MB, mulberry branch; THW, Taxilli Herba parasitizing willow; WB, willow branch; THC, Taxilli Herba parasitizing cinnamon; CB, cinnamon branch.

Figure 6

Contents of salicin in 10 batches of Taxilli Herba and its host. THM, Taxilli Herba parasitizing mulberry; MB, mulberry branch; THW, Taxilli Herba parasitizing willow; WB, willow branch; THC, Taxilli Herba parasitizing cinnamon; CB, cinnamon branch.

Figure 7

Contents of cinnamaldehyde in 10 batches of Taxilli Herba and its host. THM, Taxilli Herba parasitizing mulberry; MB, mulberry branch; THW, Taxilli Herba parasitizing willow; WB, willow branch; THC, Taxilli Herba parasitizing cinnamon; CB, cinnamon branch.

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