# Study on Quality Control Method of Taxilli Herba Based on Qualitative and Quantitative Detection of Quercitrin and 1-Deoxynojirimycin

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**Abstract** [**Objectives**] This study was conducted to establish a quality control method for mulberry-parasitizing *Taxillus chinensis* by detecting both quercitrin, an inherent component of the medicinal material, and 1-deoxynojirimycin (1-DNJ), a characteristic component derived from the mulberry host, using a "dual-substance detection" approach. [**Methods**] Ten batches of mulberry-parasitizing *T. chinensis* samples from different producing areas were collected, and *T. chinensis* samples parasitizing oil-tea camellia and oleander were used as non-mulberry host controls. TLC was used for qualitative identification of quercitrin and 1-DNJ in the samples. HPLC-UV was employed for quantitative determination of quercitrin, and HPLC-ELSD was used for quantitative determination of 1-DNJ. [**Results**] Under UV light at 365 nm, distinct quercitrin spots ($Rf = 0.46$) were observed in all ten batches of mulberry-parasitizing *Taxillus chinensis* samples and in the two control samples (*Taxillus chinensis* parasitizing oil-tea camellia and oleander). In contrast, no corresponding quercitrin spots were detected in the host samples (mulberry branches, oil-tea camellia branches, and oleander branches). Under natural light, distinct 1-DNJ spots ($Rf = 0.324$) were observed in all ten batches of mulberry-parasitizing *T. chinensis* samples and in their host mulberry branches. In contrast, no corresponding 1-DNJ spots were detected in *T. chinensis* samples parasitizing oil-tea camellia or oleander, nor in their host branches (oil-tea camellia branches and oleander branches). The average recovery of quercitrin was 99.5% ($RSD = 2.95\%$). The average quercitrin content in the ten batches of mulberry-parasitizing *T. chinensis* samples ranged from 1.98 to 3.11 mg/g, while no quercitrin was detected in the host mulberry branch samples. The average recovery of 1-DNJ was 98.03% ($RSD = 1.15\%$). The 1-DNJ content in the ten batches of mulberry-parasitizing *T. chinensis* samples ranged from 1.35 to 5.08 mg/g, while that in the host mulberry branches ranged from 3.21 to 9.41 mg/g. No 1-DNJ was detected in *T. chinensis* samples parasitizing oil-tea camellia or oleander, nor in their host branches (oil-tea camellia branches and oleander branches). [**Conclusions**] The detection method based on the "dual-component" approach using quercitrin and 1-DNJ is highly specific and simple to operate. It enables both the identification of the host origin and the quality control of mulberry-parasitizing *T. chinensis*. This "dual-component" quality control method has significant implications for guiding the quality control of parasitic herbs derived from other host plants.

**Key words** *Taxillus chinensis*; Quercitrin; 1-Deoxynojirimycin; Qualitative and quantitative detection; Quality control

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Taxilli Herba is a commonly used traditional Chinese medicine. It refers to the dried, leafy stem of *Taxillus chinensis* (DC.) Danser, a plant of the family Loranthaceae[1]. As a semi-parasitic medicinal herb, *T. chinensis* can parasitize not only mulberry trees, but also a variety of other host plants, including peach, plum, longan, lychee, carambola, oil-tea camellia, tung tree, rubber tree, banyan, kapok, as well as masson pine and Chinese cypress[2]. In addition to specifying the botanical origin of Taxilli Herba as *Taxillus chinensis* (DC.) Danser, The *Chinese Pharmacopoeia* has relatively broad requirements regarding its host plant sources. It only mandates a test to ensure the absence of cardiac glycosides, meaning that Taxilli Herba cannot be derived from hosts containing cardiac glycosides. Consequently, Taxilli Herba is a medicinal material that can originate from multiple host plant sources.

Numerous studies have shown that host plants not only provide water and inorganic salts to *T. chinensis*, but also transfer host-specific secondary metabolites to the parasitic plant, where they accumulate. This may consequently affect the quality of Taxilli Herba to varying degrees[3-8]. Particularly, the transfer of toxic components from poisonous host plants may render Taxilli Herba toxic as well[9-10]. In ancient times, people used a naming system based on the host plant to prevent the miscollection and misuse of parasitic herbs from different hosts. Numerous classical texts not only emphasized that the parasitic herb growing on mulberry (*i.e.*, "Sang Shang Jisheng") is the authentic product, but also warned of the potential toxicity of those from non-mulberry hosts[11-15]. To date, no relevant reports have been published on

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how to identify the mulberry-host origin of Taxilli Herba or how to control its quality accordingly. This study employs a dual-component detection approach for Taxilli Herba originating from mulberry hosts. By detecting 1-deoxynojirimycin (1-DNJ), a characteristic component of the mulberry host, and quercitrin, an inherent component of the parasitic herb itself, the method enables both the identification of mulberry host origin and effective quality control of Taxilli Herba.

## Materials and Methods

### Instruments and reagents

**Instruments** Agilent 1260 high-performance liquid chromatograph, evaporative light scattering detector (ELSD 3300, Alltech); KQ-5200B ultrasonic cleaner (Kun Shan Ultrasonic Instruments Co., Ltd., Jiangsu); SQP one-ten-thousandth analytical balance (Sartorius Scientific Instruments Co., Ltd.); electrothermal constant-temperature water bath (Beijing Ever Bright Medical Treatment Instrument Co., Ltd.); electrothermal blast drying oven (Shanghai Yiheng Technology Instrument Co., Ltd., model: DHG9240A); 16K benchtop centrifuge (Zhuhai Hema Medical Instrument Co., Ltd.); Mingche D24 UV pure water/ultrapure water integrated system (Merck Millipore, Germany); laboratory ultrapure water system (Guangxi Nanning Bomei Biotechnology Co., Ltd., model: Direct-Q5UV); silica gel GF254 plate, high-efficiency silica gel G (Qingdao Haiyang Chemical Co., Ltd.); 0.45 μm microporous membrane.

**Reagents** The following reagents were used: Quercitrin reference standard (Sichuan Weikeqi Biological Technology Co., Ltd., batch No.: wkq16080402, purity ≥ 98%), 1-DNJ reference standard (Chengdu Refmedic Technology Co., Ltd., batch No.: RP180921, purity ≥98%), methanol (Fisher Scientific, chromatographic grade), acetonitrile (Fisher Scientific, chromatographic grade), and ultrapure water. All other chemical reagents were of analytical grade.

The experimental materials included *T. chinensis* parasitizing mulberry and those parasitizing non-mulberry hosts. The original plants of the medicinal materials were identified by Professor Li Yonghua from the College of Pharmacy, Guangxi University of Chinese Medicine, as *T. chinensis* (DC.) Danser of the genus *Taxillus* in the family Loranthaceae. The host plants were identified as mulberry (*Morus alba* L.) of the genus *Morus* in the family Moraceae, oil-tea camellia (*Camellia oleifera* Abel.) of the genus *Camellia* in the family Theaceae, and oleander (*Nerium indicum* Mill.) of the genus *Nerium* in the family Apocynaceae. Detailed information on the experimental materials is presented in Table 1.

**Table 1 Information related to experimental materials**

| No. | Sample name | | Producing area | Collection time |
|---|---|---|---|---|
| | *T. chinensis* | Host | | |
| 1 | Mulberry-parasitizing *T. chinensis* | Mulberry branch | Cangwu County, Wuzhou City | January 2024 |
| 2 | Mulberry-parasitizing *T. chinensis* | Mulberry branch | Jinxiu County, Laibin City | February 2024 |
| 3 | Mulberry-parasitizing *T. chinensis* | Mulberry branch | Fuchuan County, Hezhou City | February 2024 |
| 4 | Mulberry-parasitizing *T. chinensis* | Mulberry branch | Debao County, Baise City | January 2024 |
| 5 | Mulberry-parasitizing *T. chinensis* | Mulberry branch | Xingye County, Yulin City | January 2024 |
| 6 | Mulberry-parasitizing *T. chinensis* | Mulberry branch | Cenxi City, Guangxi | February 2024 |
| 7 | Mulberry-parasitizing *T. chinensis* | Mulberry branch | Rongxian County, Yulin City | January 2024 |
| 8 | Mulberry-parasitizing *T. chinensis* | Mulberry branch | Qinbei District, Qinzhou City | February 2024 |
| 9 | Mulberry-parasitizing *T. chinensis* | Mulberry branch | Qinnan District, Qinzhou City | February 2024 |
| 10 | Mulberry-parasitizing *T. chinensis* | Mulberry branch | Qingxiu District, Nanning City | February 2024 |
| 11 | Oil-tea camellia-parasitizing *T. chinensis* | Oil-tea camellia branch | Wuming County, Nanning City | January 2024 |
| 12 | Oleander-parasitizing *T. chinensis* | Oleander branch | Dongxing city, Guangxi | March 2024 |

### Methods

**Sample collection and pretreatment** The collected Taxilli Herba and the tender branches of the three host plants (mulberry, oil-tea camellia), and oleander, were dried in the shade, and ground into powder, which was sieved through a No. 4 sieve for later use.

**Preparation of reference standard solutions** An accurate amount of 6.34 mg of quercitrin reference standard was weighed and added in a 10 ml volumetric flask. The solution was diluted to constant volume with methanol, and shaken to mix well to obtain a quercitrin reference standard solution at a concentration of 634.00 mg/L.

An accurate amount of 15.25 mg of 1-DNJ reference standard was weighed and added in a 10 ml volumetric flask. The solution was diluted to constant volume with methanol, and shaken to mix well to obtain a 1-DNJ reference standard solution at a concentration of 1 525.00 mg/L.

### Thin-layer chromatography (TLC)

**TLC for quercetin**[16]

(1) Preparation of test solutions: An accurate amount of 1.0 g of each medicinal material powder (sieved through a No. 4 sieve) was weighed, and added with 80% methanol. The mixture was ultrasonicated for 45 min, and then filtered. The residue was further extracted with the same volume of 80% methanol under ultrasonication for another 45 min. The two filtrates were combined, concentrated by rotary evaporation, and diluted to 5 ml with 80% methanol to obtain a test solution.

(2) Qualitative TLC detection method for quercitrin: High-performance silica gel G plates were used as the TLC plates. The developing solvent was a mixture of butyl acetate, cyclohexane, formic acid, and water (5∶1.2∶1.8∶0.3, *V/V/V/V*). A 3% aluminum chloride ethanol solution was used as the chromogenic agent. The sample application volume was 1 μl. After development, the plates were air-dried and examined under a UV lamp at 365 nm.

**TLC for 1-DNJ**[17-19]

(1) Preparation of test solution: An accurate amount (1.0 g) of the medicinal material powder (sieved through a No. 4 sieve) was weighed, and added with 100 ml of 50% ethanol. The mixture was refluxed for 2 h, and then filtered. The residue was further extracted with the same volume of 50% ethanol under reflux for another 2 h. The two filtrates were combined, and the ethanol was removed by rotary evaporation. The extract was sieved through a 732 hydrogen-type strong acid cation exchange resin, and eluted with ammonia water, and the eluate was evaporated to dryness by rotary evaporation. The residue was dissolved and diluted to 1 ml with methanol to obtain the test solution.

(2) Qualitative TLC detection method for 1-DNJ: Silica gel GF254 TLC plates were used as the stationary phase. The developing solvent consisted of n-butanol, methanol, and ammonia water (4∶10∶1.8, *V/V/V*). A o-tolidine chlorine reagent solution was used as the chromogenic agent. The sample application volume was 1 μl. After development, the plates were examined under natural light.

**Quantitative detection method by high-performance liquid chromatography (HPLC)**

**HPLC quantitative detection method for quercitrin**[20-21]

(1) Preparation of test solution: An accurate amount (0.50 g) of mulberry-parasitizing *T. chinensis* powder was weighed and placed in a 100 ml conical flask with a stopper. Next, 25 ml of 75% methanol was added, and the flask was weighed. The mixture was ultrasonicated for 45 min, and then cooled, and the lost weight was supplemented. The solution was filtered through a 0.45 μm microporous membrane to obtain the test solution.

(2) Chromatographic conditions and system suitability: The chromatographic separation was performed on a Waters-$C_{18}$ column (5 μm, 4.6 mm × 250 mm) with a column temperature maintained at (30 ± 5) ℃. Gradient elution was carried out using mobile phase A (acetonitrile) and mobile phase B (0.1% phosphoric acid) as follows: 0 – 45 min, 18% – 32% A. The flow rate was 1.0 ml/min, and the injection volume was 10 μl. The detection wavelength was 254 nm.

(3) Linearity investigation: The quercitrin reference standard solution was diluted with methanol to prepare working solutions at concentrations of 6.34, 12.68, 25.36, 126.80, 317.00, and 634.00 mg/L for linearity testing. An aliquot (10 μl) of each solution was injected, and the peak areas were measured. Regression analysis was performed with concentration as the abscissa and peak area as the ordinate. The resulting regression equation for quercitrin was $Y = 2.64 \times 10^7 X + 4\,947.87$, with a correlation coefficient $r = 0.999\,9$, indicating a good linear relationship between concentration and peak area within the range of 6.34 – 634.00 mg/L.

(4) Precision test: The quercitrin reference standard solution was precisely injectedfor 6 times in succession under the specified chromatographic conditions. The average peak area of quercitrin was calculated, and the relative standard deviation (*RSD*) was 0.18%, which meets the requirements for quantitative analysis of the *Chinese Pharmacopoeia*.

(5) Stability test: Six portions of mulberry-parasitizing *T. chinensis* powder, each weighing 0.50 g, were accurately weighed. Test solutions were prepared in parallel according to the method for quercitrin test solutions. Under the specified chromatographic conditions, the quercitrin content in the test solutions was determined once every 5 h over a 24-h period. The relative standard deviation (*RSD*) for quercitrin was 0.89%, indicating that quercitrin remained stable in the test solutions within 24 h.

(6) Repeatability test: Six portions of mulberry-parasitizing *T. chinensis* powder, each weighing 0.5 g, were accurately weighed. Test solutions were prepared in parallel according to the method for quercitrin test solutions. Under the specified chromatographic conditions, the quercitrin content in the test solutions was determined. The relative standard deviation (*RSD*) for quercitrin was 0.64%, indicating good repeatability of the method.

(7) Recovery test: Six portions of mulberry-parasitizing *T. chinensis* (THM-1) powder with a known quercitrin content (2.29 mg/g) were accurately weighed. A precise amount of 567.00 μg of quercitrin reference standard was added to each portion. Test solutions were prepared in parallel according to the method for quercitrin test solutions. Under the specified chromatographic conditions, the quercitrin content was calculated based on peak areas. The average recovery of quercitrin was 99.51%, with a relative standard deviation (*RSD*) of 2.86%, indicating good repeatability of the method. The results are shown in Table 2.

**Table 2 Test results of quercitrin recovery from mulberry-parasitizing *T. chinensis*** ($n = 6$)

| No. | Original amount // μg | Added amount // μg | Measured amount // μg | Recovery // % | Average recovery // % | *RSD* // % |
|---|---|---|---|---|---|---|
| 1 | 567.28 | 567.00 | 1 161.88 | 104.87 | 99.51 | 2.86 |
| 2 | 568.79 | 567.00 | 1 121.88 | 97.55 | | |
| 3 | 578.10 | 567.00 | 1 143.69 | 99.75 | | |
| 4 | 571.83 | 567.00 | 1 137.55 | 99.77 | | |
| 5 | 570.66 | 567.00 | 1 122.41 | 97.31 | | |
| 6 | 575.41 | 567.00 | 1 129.90 | 97.79 | | |

**HPLC quantitative detection method for 1-DNJ[22-23]**

(1) Preparation of test solution: An accurate amount of 1.00 g of mulberry-parasitizing *T. chinensis* powder was weighed and added in a 250 ml conical flask with a stopper. Next, 100 ml of 50% ethanol was added, and the mixture was refluxed for 2 h, and then filtered. The residue was further extracted with the same volume of 50% ethanol under reflux for another 2 h. The two filtrates were combined, and the ethanol was removed by rotary evaporation. The extract was loaded to 732 hydrogen-type strong acid cation exchange resin, and eluted with ammonia water, and the eluate was evaporated to dryness by rotary evaporation. The residue was dissolved and diluted to 5 ml with methanol to obtain the test solution.

(2) Chromatographic conditions and system suitability: The chromatographic separation was performed on an Agilent-$C_{18}$ amino column (5 μm, 4.6 mm × 250 mm) with a column temperature maintained at (30 ± 5) ℃. The flow rate was 0.5 ml/min, and the injection volume was 10 μl. The analysis time was 15 min. The mobile phase consisted of acetonitrile-water (90 ∶ 10, *V/V*) with a gradient elution from 82% C to 68% C. The evaporative light scattering detector (ELSD) was set with following parameters: carrier gas flow rate 3.0 L/min, temperature 90 ℃, and gain 2.

(3) Linearity investigation: The 1-DNJ reference standard solution was diluted with methanol to prepare working solutions at concentrations of 15.25, 61.00, 152.50, 305.00, 762.50, and 1525.00 mg/L for linearity testing. An aliquot (10 μl) of each solution was injected, and the peak area was measured. Regression analysis was performed with concentration as the abscissa and peak area as the ordinate. The resulting regression equation for 1-DNJ was $Y = 926.85X - 755.42$, with a coefficient of determination $r^2 = 0.999$, indicating a good linear relationship between concentration and peak area within the range of 15.25 to 1 525.00 mg/L.

(4) Precision test: The 1-DNJ reference standard solution was precisely injected for 6 times in succession under the specified chromatographic conditions. The average peak area of 1-DNJ was calculated, and the relative standard deviation (*RSD*) was 0.29%, which meets the requirements for quantitative analysis of the *Chinese Pharmacopoeia*.

(5) Stability test: Six portions of mulberry-parasitizing *T. chinensis* powder, each weighing 0.50 g, were accurately weighed. Test solutions were prepared in parallel according to the method for 1-DNJ test solutions. Under the specified chromatographic conditions, the 1-DNJ content in the test solutions was determined once every 5 h over a 24-h period. The relative standard deviation (*RSD*) was 1.76%, indicating that 1-DNJ remained stable in the test solution within 24 h.

(6) Repeatability test: Six portions of mulberry-parasitizing *T. chinensis* powder, each weighing 0.5 g, were accurately weighed. Test solutions were prepared in parallel according to the method for 1-DNJ test solutions. Under the specified chromatographic conditions, the 1-DNJ content in the test solutions was determined. The relative standard deviation (*RSD*) was 2.78%, indicating good repeatability of the method.

(7) Recovery test: Six portions of mulberry-parasitizing *T. chinensis* (THM-1) powder with a known 1-DNJ content (2.19 mg/g) were accurately weighed. A precise amount (1.00 mg) of 1-DNJ reference standard was added to each portion. Test solutions were prepared in parallel according to the method for 1-DNJ test solutions. Under the specified chromatographic conditions, the 1-DNJ content was calculated based on peak areas. The average recovery of 1-DNJ was 98.03%, with a relative standard deviation (*RSD*) of 1.15%, indicating good repeatability of the method. The results are shown in Table 3.

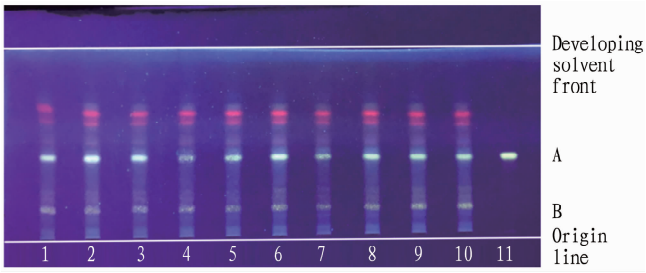
**Table 3 Results of recovery test for 1-DNJ in mulberry-parasitizing *T. chinensis* ($n = 6$)**

| No. | Original amount//μg | Added amount//μg | Measured amount//μg | Recovery//% | Average recovery//% | *RSD*//% |
|---|---|---|---|---|---|---|
| 1 | 1.05 | 1.00 | 2.05 | 99.59 | 98.03 | 1.15 |
| 2 | 1.06 | 1.00 | 2.04 | 97.88 | | |
| 3 | 1.09 | 1.00 | 2.07 | 97.70 | | |
| 4 | 1.10 | 1.00 | 2.08 | 97.87 | | |
| 5 | 1.07 | 1.00 | 2.06 | 98.86 | | |
| 6 | 1.16 | 1.00 | 2.12 | 96.29 | | |

## Results and Analysis

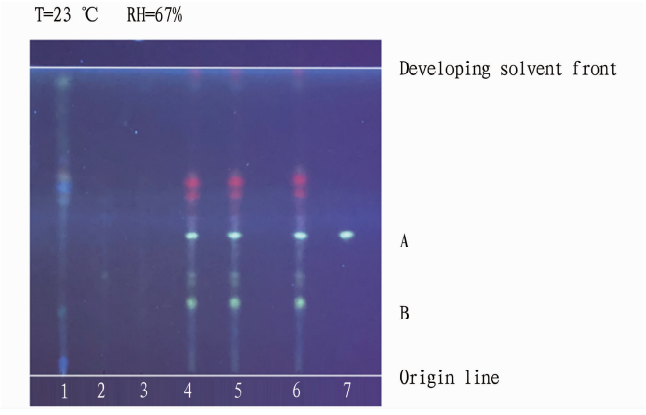
**Identification results of quercitrin by thin-layer chromatography (TLC)**

According to the TLC identification method for quercitrin, aliquots (1 μl each) of the test solutions of mulberry-parasitizing *T. chinensis*, oil-tea camellia-parasitizing *T. chinensis*, oleander-parasitizing *T. chinensis*, and their respective host samples from 10 different batches were applied onto two high-performance silica gel G plates. The results showed that spots of the same color appeared at positions corresponding to the quercitrin reference standard for mulberry-parasitizing *T. chinensis*, oil-tea camellia-parasitizing *T. chinensis*, and oleander-parasitizing *T. chinensis*. In contrast, no corresponding spots were observed for mulberry branches, oil-tea camellia branches, or oleander branches at the position of the quercitrin reference standard, indicating that these host branches do not contain quercitrin and that quercitrin is an inherent component of Taxilli Herba. The results are shown in Fig. 1 and Fig. 2.




1 – 10: Ten batches of mulberry-parasitized *T. chinensis* from different producing areas; 11: Quercitrin reference solution; A: Yellowish-green spot (quercitrin).

**Fig. 1 Figure TLC of mulberry-parasitized *T. chinensis* samples from ten producing areas**

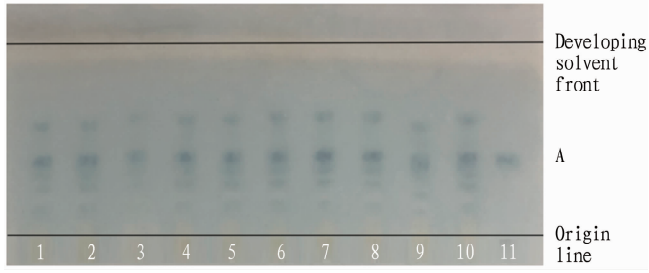



1. Mulberry branch; 2. Oil-tea camellia branch; 3. Oleander branch; 4. Mulberry-parasitizing *T. chinensis*; 5. Oil-tea camellia-parasitizing *T. chinensis*; 6. Oleander-parasitizing *T. chinensis*; 7. Quercitrin.
A. Greenish-yellow spot (quercitrin).

**Fig. 2 TLC chromatogram of *T. chinensis* samples parasitizing mulberry, oil-tea camellia and oleander, and respective host samples**

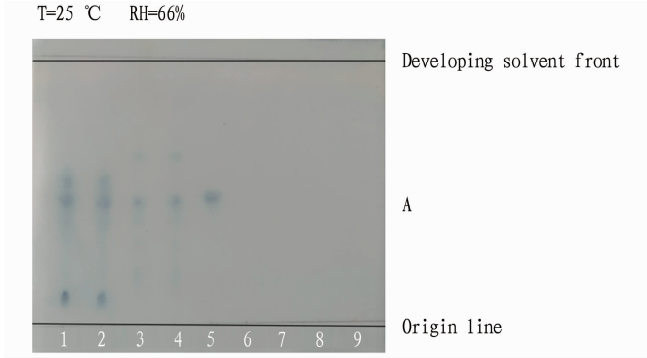
## Identification results of 1-DNJ by TLC

According to the TLC method for 1-DNJ, aliquots (1 μl each) of the test solutions of mulberry-parasitizing *T. chinensis*, oil-tea camellia-parasitizing *T. chinensis*, oleander-parasitizing *T. chinensis*, and their respective host samples from 10 different batches were applied onto two silica gel GF254 TLC plates. The results are shown in Fig. 3 and Fig. 4. All ten batches of Taxilli Herba test solutions showed spots of the same color at positions corresponding to the 1-DNJ reference standard on the chromatogram, indicating that all ten batches contained 1-DNJ. As shown in Fig. 4, mulberry branches and mulberry-parasitizing *T. chinensis* also exhibited spots of the same color at positions corresponding to the 1-DNJ reference standard, indicating that both mulberry branches and mulberry-parasitizing *T. chinensis* contain 1-DNJ. In contrast, oil-tea camellia branches, oil-tea camellia-parasitizing *T. chinensis*, oleander branches, and oleander-parasitizing *T. chinensis* showed no corresponding spots at the position of the 1-DNJ reference standard, indicating that these samples do not contain 1-DNJ. Therefore, 1-DNJ is a characteristic component of the mulberry host. The 1-DNJ detected in mulberry-parasitizing *T. chinensis* is transferred from the mulberry host. TLC identification of 1-DNJ in mulberry-parasitizing *T. chinensis* can effectively distinguish it from *T. chinensis* derived from non-mulberry hosts.




1 – 10: Ten batches of mulberry-parasitizing *T. chinensis* from different producing areas; 11: 1-DNJ.

**Fig. 3 TLC chromatogram of 1-DNJ in mulberry-parasitizing *T. chinensis* samples from ten producing areas**




1 – 2. Mulberry branch; 3 – 4. Mulberry-parasitizing *T. chinensis*; 5. 1-DNJ; 6. Oil-tea camellia-parasitizing *T. chinensis*; 7. Oil-tea camellia branch; 8. Oleander-parasitizing *T. chinensis*; 9. Oleander branch.

**Fig. 4 TLC chromatogram of 1-DNJ in mulberry-parasitizing and non-mulberry-parasitizing *T. chinensis* and their host samples**

## Determination of quercitrin content

Powdered samples of mulberry-parasitizing *T. chinensis*, oil-tea camellia-parasitizing *T. chinensis*, oleander-parasitizing *T. chinensis*, and their respective host branches were prepared. Test solutions were prepared in parallel according to the method for quercitrin test solutions. The quercitrin content was determined under the specified chromatographic conditions. The results are shown in Table 4, and the chromatogram is presented in Fig. 5.

As shown in Table 4, all samples of mulberry-parasitizing *T. chinensis* contained quercitrin, whereas the mulberry branches (host) did not. The average quercitrin content of the ten batches of mulberry-parasitizing *T. chinensis* ranged from 1.98 to 3.11 mg/g. The quercitrin content was 3.20 mg/g in oil-tea camellia-parasitizing *T. chinensis* and 2.72 mg/g in oleander-parasitizing *T. chinensis*. The host branches (oil-tea camellia and oleander) contained no quercitrin. This indicates that quercitrin in the parasitic herb is not derived from the host, but is a specific component of the medicinal material itself, qualifying it as a suitable indicator for quality control.

**Table 4 Determination results of quercitrin content in *T. chinensis* samples parasitizing mulberry, oil-tea camellia and oleander, and respective host plants**

| Sample name | Content // mg/g | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | $\overline{X}$ |
| Mulberry-parasitizing *T. chinensis* | 2.29 | 1.98 | 2.10 | 2.16 | 2.64 | 2.77 | 2.10 | 2.82 | 2.33 | 3.11 | 2.29 |
| Mulberrybranch | – | – | – | – | – | – | – | – | – | – | – |
| Oil-tea camellia-parasitizing *T. chinensis* | 3.20 | – | – | – | – | – | – | – | – | – | – |
| Oil-tea camellia branch | – | – | – | – | – | – | – | – | – | – | – |
| Oleander-parasitizing *T. chinensis* | 2.72 | – | – | – | – | – | – | – | – | – | – |
| Oleander branch | – | – | – | – | – | – | – | – | – | – | – |

The experimental results further confirm that quercitrin is an inherent component of mulberry-parasitizing *T. chinensis* and that its presence is independent of the mulberry host.

**Determination of 1-DNJ content**

Powdered samples of mulberry-parasitizing *T. chinensis* and its host mulberry branches were prepared in parallel according to the method for 1-DNJ test solutions. The 1-DNJ content was determined under the specified chromatographic conditions. The results are shown in Table 5. HPLC chromatograms of 1-DNJ in mulberry-parasitizing *T. chinensis*, non-mulberry-parasitizing *T. chinensis*, and their respective host samples are presented in Fig. 6.

**Table 5 Determination results of 1-DNJ content in in *T. chinensis* samples parasitizing mulberry, oil-tea camellia and oleander, and respective host plants**

| Sample name | 1-DNJ content // mg/g | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | $\overline{X}$ |
| Mulberry-parasitizing *T. chinensis* | 2.19 | 3.18 | 5.08 | 1.35 | 3.66 | 3.22 | 2.98 | 4.54 | 1.96 | 3.11 | 3.13 |
| Mulberry branch | 4.06 | 5.31 | 9.41 | 4.54 | 5.29 | 6.01 | 4.76 | 7.31 | 3.21 | 4.51 | 5.44 |
| Oil-tea camellia-parasitizing *T. chinensis* | – | – | – | – | – | – | – | – | – | – | – |
| Oil-tea camellia branch | – | – | – | – | – | – | – | – | – | – | – |
| Oleander-parasitizing *T. chinensis* | – | – | – | – | – | – | – | – | – | – | – |
| Oleander branch | – | – | – | – | – | – | – | – | – | – | – |

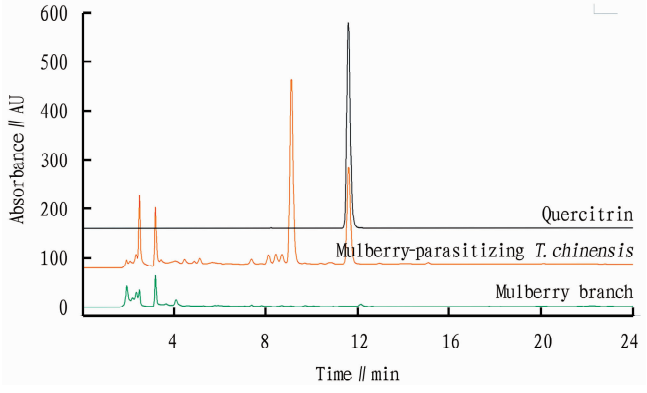



**Fig. 5 HPLC chromatogram of mulberry-parasitizing *T. chinensis*, mulberry branch and quercitrin reference standard**

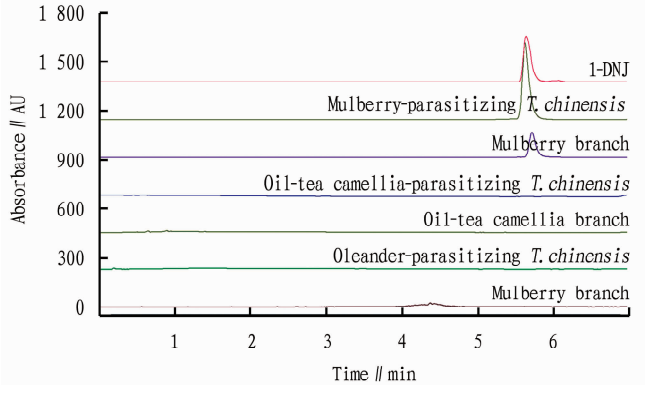



**Fig. 6 HPLC chromatogram of 1-DNJ in mulberry-parasitizing and non-mulberry-parasitizing *T. chinensis* and their host samples**

## Conclusions and Discussion

Mulberry-parasitizing *T. chinensis*, also known as "Sang Shang Jisheng" or "Sang Jisheng", was first recorded in *Shen Nong's Herbal Classic*: "Sang Shang Jisheng is used for treating lower back pain and infantile back stiffness, preventing miscarriage, nourishing the skin, strengthening hair and teeth, and promoting beard and eyebrow growth[24]." A review of historical herbal literature reveals that parasitic herbs have traditionally been named after their host plants. That is, the parasitic herb growing on mulberry is called "Sang Shang Jisheng". As recorded in *Bencao Jing Ji Zhu* (*Variorum of Shennong's Classic of Materia Medica*): "The one growing on mulberry is named Sang Shang Jisheng. Similarly, practitioners have used those growing on poplar and maple, naming them after their respective host trees[25]." In Li Shizhen's *Compendium of Materia Medica*, in addition to "Sang Shang Jisheng" (Sang Jisheng), other parasitic herbs such as "Liu Jisheng" and "Tao Jisheng" were also recorded. According to the text, Liu Jisheng is used to treat "stabbing pain in the diaphragm", while Tao Jisheng is used to treat "children poisoned by parasites, manifested as hard abdominal pain, sallow complexion,

and emaciation"[11]. In Xiao Budan's *Lingnan Caiyao Lu* (*Records of Medicinal Herbs from Lingnan*), a total of eight parasitic herbs from different host plants were documented, including mulberry-parasitizing *T. chinensis*, pine-parasitizing *T. chinensis*, Chinese tallow-parasitizing *T. chinensis*, sweetgum-parasitizing parasite, tung tree-parasitizing *T. chinensis*, sand pear-parasitizing *T. chinensis*, cypress-parasitizing *T. chinensis*, and wampee-parasitizing *T. chinensis*[26].

Taxilli Herba derived from different host plants shares the same botanical origin, making it difficult to effectively distinguish between them based on morphological or even microscopic characteristics[27]. Therefore, in ancient times, to ensure the authenticity of mulberry-parasitizing material, the *Compendium of Materia Medica* recorded that "only those collected personally or together with the mulberry branch can be used"[11]. In this study, based on the phenomenon that the mulberry host transfers its characteristic component, 1-DNJ, to the parasitic plant *T. chinensis*, where it accumulates[3], a qualitative TLC method was first used to identify 1-DNJ in mulberry-parasitizing *T. chinensis* from ten different producing areas. The results showed that all ten samples exhibited spots corresponding to the 1-DNJ reference standard. Based on the qualitative TLC detection of 1-DNJ in mulberry-parasitizing *T. chinensis*, high-performance liquid chromatography (HPLC) was further employed to quantitatively determine 1-DNJ in both the mulberry-parasitizing *T. chinensis* and its host mulberry branches. *T. chinensis* samples parasitizing oil-tea camellia and oleander were used as non-mulberry host controls. The results showed that 1-DNJ was detected in both mulberry-parasitizing *T. chinensis* and its mulberry host, but not in *T. chinensis* parasitizing oil-tea camellia or oleander, nor in their host plants. Therefore, both TLC and HPLC methods targeting 1-DNJ can effectively distinguish mulberry-parasitizing *T. chinensis* from *T. chinensis* derived from non-mulberry hosts.

The average quercitrin content in the ten batches of mulberry-parasitizing *T. chinensis* ranged from 1.98 to 3.11 mg/g, while no quercitrin was detected in the host mulberry branches. The 1-DNJ content in the ten batches of mulberry-parasitizing *T. chinensis* from different producing areas ranged from 1.35 to 5.08 mg/g. On the basis of effectively identifying the mulberry host and distinguishing mulberry-parasitizing from non-mulberry-parasitizing *T. chinensis*, this study performed qualitative and quantitative detection of quercitrin, an inherent component of the parasitic herb. This approach differs from the qualitative detection of quercetin prescribed by the *Chinese Pharmacopoeia* for Taxilli Herba. Studies have shown that quercetin in Taxilli Herba is primarily derived from the acid hydrolysis of quercitrin, indicating that quercetin mainly exists in the form of quercitrin in nature, with only a very small amount of naturally occurring quercetin[21]. Both quercitrin and quercetin belong to the flavonoid family and exhibit strong biological activities[28-29]. Therefore, in this study, quercitrin was used as a quality control indicator for mulberry-parasitizing *T. chinensis*. Both qualitative and quantitative detection methods for quercitrin were established using TLC and HPLC, respectively. The TLC results showed that samples of non-mulberry-parasitizing *T. chinensis* from ten different producing areas, as well as those parasitizing oil-tea camellia and oleander, exhibited spots corresponding to the quercitrin reference standard. The results from both HPLC and TLC showed that quercitrin could be detected in mulberry-parasitizing *T. chinensis*, oil-tea camellia-parasitizing *T. chinensis*, and oleander-parasitizing *T. chinensis*, but could not be detected in any of the three host plants. These findings indicate that quercitrin is a specific component of the parasitic herb itself.

This study established a "dual-component" quality control method based on the detection of 1-DNJ and quercitrin. The method is highly specific, simple to operate, and enables both the identification of the mulberry host origin and the quality control of mulberry-parasitizing *T. chinensis*. This "dual-component" approach has significant implications for guiding the quality control of parasitic herbs derived from other host plants.

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