Analysis by RP-HPLC of Mangiferin Component Correlation between Medicinal Loranthus and their Mango Host Trees

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This study proposes a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the determination of mangiferin in medicinal loranthus shrubs and their mango or non-mango host trees. Mangiferin in samples was extracted with a solution of 40% methanol. Analytical determination was conducted by RP-HPLC with ultraviolet detection at 258 nm. Chromatographic separation was achieved on an Inertsil ODS-SP column (250 × 4.6 mm, 5 μm) by isocratic elution with methanol–0.1% aqueous phosphoric acid (31:69, v/v). Mangiferin contents were 5.04 to 18.95 mg/g in mango trees and 0.44 to 3.72 mg/g in medicinal loranthus parasitized on mango host trees. Mangiferin could not be detected in non-mango trees and their loranthus shrubs. This study indicated that host trees could affect the quality of medicinal loranthus by transporting host-inherent components into loranthus. Salicin can be detected in loranthus parasitized on mulberry and willow, implying that DNJ and salicin, as host-inherent secondary metabolites, can be transported into their loranthus shrubs (14, 15, 16). In this study, the mangiferin contents of loranthus parasitized on mango (Mangifera indica L.) and non-mango trees (Melia azedarach L.) were analyzed using reversed-phase high-performance liquid chromatography (RP-HPLC). After observing the mangiferin contents of loranthus parasitized on mango and non-mango host trees, some experimental evidence is presented about the effects of host trees on the quality of loranthus.

Materials and Methods

Materials
The standard substance of mangiferin (98% pure, lot number 111607-200402) was purchased from the National Institute for the Control of Pharmaceuticals and Biological products (Beijing, China). Methanol (HPLC grade) was purchased from Labscience, Inc. Ultrapure water was used. All other materials and solvents were analytical grade. The samples of loranthus parasitized on mango and non-mango trees were collected from Guangxi Province in January of 2012. The species of loranthus was identified by Professor Huaxing Qiu (South China Botanical Garden, Chinese Academy of Sciences) as Taxillus chinensis (DC.) Danser. The species of host trees were identified as Mangifera indica L. and Melia azedarach L., respectively. All samples were washed and dried. The branches and leaves were separately mashed and sieved (60 mesh) to powder for use.

Instrumentation
The chromatographic system consisted of an LC-10AT HPLC (Shimadzu, Kyoto, Japan), an SPD-20A UV detector (Shimadzu) and an Inertsil ODS-SP column (Shimadzu; 250 × 4.6 mm, 5 μm).

HPLC conditions
The mobile phase was methanol–aqueous 0.1% phosphoric acid (31:69, v/v). The analyses were conducted at a flow rate of 1.0 mL/min with ultraviolet (UV) detection at 258 nm (17, 18). The operation was conducted at room temperature.

Introduction
Loranthus, a semi-parasitic shrub from the family Loranthaceae, is a well known herb used in China for liver and kidney reinforcement, tendon and bone strengthening, relief of rheumatic conditions and the prevention of abortion. The Chinese Pharmacopoeia indicates that the official species of medicinal loranthus is Taxillus chinensis (DC.) Danser (1). Many different host species in nature can be parasitized by this semi-parasitic shrub (2). However, no restrictions are placed on the host trees of medicinal loranthus in the Chinese Pharmacopoeia. As a traditional Chinese medicine (TCM), medicinal loranthus comes from a very broad range of hosts, such as mulberry, willow, poplar, peach, pine, mango and wampee trees. Phytochemical analysis has revealed the presence of glycosides, alkaloids, flavonoids, resins, proteins, carbohydrates, reducing sugars, saponins, steroids, terpenoids and tannins in loranthus (3). So far, there are few documents regarding the effects of host trees on the phytochemical compositions and activities of loranthus. These documents indicate that the compositions of loranthus are host-dependent in degree, but not in kind (4, 5).

Mangiferin (Figure 1) is a xanthone compound found in many species of plants, such as Mangifera indica, Anemarrhena asphodeloides and Belamcanda chinensis, with anti-inflammatory (6), antioxidant (7), antiviral (8) and antitumor activity (9) and other pharmacological activities (12, 13), and can be used for the treatment of diabetes (10) or for myocardial preservation (11). A previous study showed that host specific secondary metabolites 1-deoxynojirimycin (DNJ) and salicin can be detected in loranthus parasitized on mulberry and willow, implying that DNJ and salicin, as host-inherent secondary metabolites, can be transported into their loranthus shrubs (14, 15, 16). In this study, the mangiferin contents of loranthus parasitized on mango (Mangifera indica L.) and non-mango trees (Melia azedarach L.) were analyzed using reversed-phase high-performance liquid chromatography (RP-HPLC). After observing the mangiferin contents of loranthus parasitized on mango and non-mango host trees, some experimental evidence is presented about the effects of host trees on the quality of loranthus.
Preparation of standard solutions
The stock solution of mangiferin standard substance was pre-pared in methanol at a concentration of 477.60 \( \text{mg/mL} \). The working standard solutions, with concentrations of 0.76, 3.82, 19.10, 95.52 and 477.60 \( \text{mg/mL} \), were prepared in methanol by diluting the stock solution of mangiferin. The stock solution was kept at 4 \( ^\circ \text{C} \) in a refrigerator before use.

Preparation of sample solutions
A total of 0.12 g of the sample was added to 20 mL of 40% methanol solution. The sample was ultrasonically extracted for 30 min and cooled. All sample solutions were filtered through a 0.45 \( \mu \text{m} \) microporous membrane.

Validation of the HPLC method
To assess the limits of detection (LOD) and quantification (LOQ), the dilute solution of mangiferin standard substance was further diluted to a series of concentrations with methanol. The LOD and LOQ were determined as signal-to-noise (S/N) ratios of 3 and 10, respectively.

Results and Discussion
Linear relationship and method validation
A total of 5 \( \mu \text{L} \) of standard working solution was injected into the HPLC system to determine the peak areas. The chromatogram of the mangiferin standard substance is shown in Figure 2. The peak areas and mangiferin concentrations were analyzed by regression methods and calibration curves were drawn to calculate the regression equation. The regression equation was \( Y = 27176X - 5645.6 \), with a correlation coefficient \( (r) \) of 1.0000. As a result, a good linear relationship was observed between the concentrations and their corresponding peak areas when the mangiferin ranged from concentrations of 0.76 to 477.60 \( \mu \text{g/mL} \). The LOD was 0.21 \( \mu \text{g/mL} \) and the LOQ was 0.63 \( \mu \text{g/mL} \). Thus, the proposed method is sufficiently sensitive to analyze mangiferin.

Precision testing
A total of 5 \( \mu \text{L} \) of the same sample solution was injected into the HPLC system six times to determine mangiferin contents. The relative standard deviation (RSD) was 0.98% \( (n = 6) \), which proved the precision of the equipment.

Stability testing
Under normal room temperature, 5 \( \mu \text{L} \) of the same sample solution was injected seven times into the HPLC system at 0, 2, 4, 6, 8, 12 and 24 h to determine mangiferin contents. The RSD was 0.95% \( (n = 7) \), showing that the mangiferin sample solution was stable within 24 h.

Reproducibility testing
Six samples were accurately weighed. The mangiferin content was determined after extraction. The RSD was 1.80% \( (n = 6) \), indicating the reproducibility of the present method.

Average recovery rate testing
Five portions of the accurately weighed sample with previously determined mangiferin contents were added to a certain amount of the mangiferin standard substance. After extraction and injection, the mangiferin contents of these portions were re-determined and showed an average recovery rate of 98.34% and RSD of 1.38% (Table I), proving that the current method had a high recovery rate and met the experimental requirements.

Determination of mangiferin content in samples
A total of 5 \( \mu \text{L} \) of sample solution was injected into the HPLC system to determine mangiferin contents. The results are shown in Figure 3 and Table II.

Relative mangiferin contents between loranthus herbs and their host trees
The medicinal loranthus recorded in the Chinese Pharmacopoeia comprises the dry leaves and branches of *Taxillus chinensis* (DC.) Danser. The relative mangiferin

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**Table I**

<table>
<thead>
<tr>
<th>Sample weight (g)</th>
<th>Contents of mangiferin (mg)</th>
<th>Amount of added standards (mg)</th>
<th>Measured value (mg)</th>
<th>Recovery rate (%)</th>
<th>Average recovery rate (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0626</td>
<td>0.7800</td>
<td>0.7644</td>
<td>1.5426</td>
<td>99.76</td>
<td>98.34</td>
<td>1.38</td>
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<td>0.0613</td>
<td>0.7638</td>
<td>0.7644</td>
<td>1.5161</td>
<td>98.41</td>
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<tr>
<td>0.0611</td>
<td>0.7613</td>
<td>0.7644</td>
<td>1.5146</td>
<td>98.55</td>
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<td></td>
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<tr>
<td>0.0603</td>
<td>0.7513</td>
<td>0.7644</td>
<td>1.4848</td>
<td>95.95</td>
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</tr>
<tr>
<td>0.0608</td>
<td>0.7576</td>
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<td>99.90</td>
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<td></td>
</tr>
<tr>
<td>0.0611</td>
<td>0.7613</td>
<td>0.7644</td>
<td>1.5063</td>
<td>97.46</td>
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</tr>
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</table>
Contents between loranthus shrubs and their host trees are listed in Table II. The results showed that the mangiferin contents of loranthus parasitized on mango and their host trees (Mangifera indica L.) were 0.44 to 3.72 mg/g and 5.04 to 18.95 mg/g, respectively. Clearly, the mangiferin contents of the herb branches were higher than the leaves of loranthus parasitized on mango host trees, whereas the results of the host trees indicated the opposite. The mangiferin content ratios in the leaves of loranthus/mango host trees were 3.30 to 3.93%, and the results of branches were 23.11 to 31.40%. This

### Table II

<table>
<thead>
<tr>
<th>Number</th>
<th>Host tree</th>
<th>Collection sites</th>
<th>Samples</th>
<th>Sample weight (g)</th>
<th>Contents of mangiferin (mg/g)</th>
<th>Relative contents* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mangifera indica</td>
<td>Nanning</td>
<td>Branches of Mangifera indica</td>
<td>0.1232</td>
<td>5.04</td>
<td>25.76</td>
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<tr>
<td></td>
<td></td>
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<td>0.1254</td>
<td>13.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Branches of Taxillus chinensis</td>
<td>0.1230</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leaves of Taxillus chinensis</td>
<td>0.1265</td>
<td>0.44</td>
<td>3.31</td>
</tr>
<tr>
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<td>Mangifera indica</td>
<td>Nanning</td>
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<td>0.1230</td>
<td>6.14</td>
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<td>17.11</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Branches of Taxillus chinensis</td>
<td>0.1238</td>
<td>1.93</td>
<td>31.40</td>
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<td></td>
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<td>Leaves of Taxillus chinensis</td>
<td>0.1244</td>
<td>0.62</td>
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</tr>
<tr>
<td>3</td>
<td>Mangifera indica</td>
<td>Qinzhou</td>
<td>Branches of Mangifera indica</td>
<td>0.1203</td>
<td>12.46</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Leaves of Mangifera indica</td>
<td>0.1211</td>
<td>18.95</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td>Branches of Taxillus chinensis</td>
<td>0.1240</td>
<td>3.72</td>
<td>29.83</td>
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<td>Leaves of Taxillus chinensis</td>
<td>0.1205</td>
<td>0.63</td>
<td>3.30</td>
</tr>
<tr>
<td>4</td>
<td>Mangifera indica</td>
<td>Qinzhou</td>
<td>Branches of Mangifera indica</td>
<td>0.1248</td>
<td>6.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>14.33</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Branches of Taxillus chinensis</td>
<td>0.1203</td>
<td>1.39</td>
<td>23.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leaves of Taxillus chinensis</td>
<td>0.1231</td>
<td>0.56</td>
<td>3.93</td>
</tr>
<tr>
<td>5</td>
<td>Melia azedarach</td>
<td>Fangchenggang</td>
<td>Leaves of Mangifera indica</td>
<td>0.1219</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leaves of Taxillus chinensis</td>
<td>0.1223</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

*Relative content (%): (content of mangiferin in parasitic loranthus) / (content of mangiferin in host tree) × 100%.
difference may imply that the transportation of this compound is attributable to the physical connection between the two plants; however, further study is required to address this possibility. Moreover, no mangiferin could be detected in loranthus parasitized on non-mango and host trees (Melia azedarach L.).

**Evaluation of effects of host trees on the quality of loranthus**

It has been reported that mangiferin is a specific xanthone, a chemical compound found in mango trees (19). The current results of mangiferin contents in mango and non-mango trees also indicate that mangiferin is a specific inherent component in mango trees. Therefore, loranthus herb containing mangiferin components is most likely attributable to its parasitization on mango host trees, because in this study, mangiferin components could not be found in loranthus parasitized on non-mango trees (Melia azedarach L.). These experimental results are consistent with the authors’ earlier reports on DNJ and salicin, components that are specific to mulberry or willow host trees, which could clearly be detected from their respective parasitised loranthus. The present results indicate that host trees are likely the key factors affecting the quality of medicinal loranthus. Therefore, it is necessary to study the pharmacological efficacy of medicinal loranthus from different host trees.

**Quality control of medicinal loranthus**

Given that mangiferin is an inherent component in mango trees, and that medicinal loranthus contains mangiferin components due to these loranthus from mango host trees, the methods established in this study can be used to control the quality of medicinal loranthus from mango host trees and to distinguish medicinal loranthus from non-mango host trees.

**Conclusion**

As an inherent component and secondary metabolite, mangiferin in mango host trees can be transported into the medicinal loranthus herbs that parasite these trees. Therefore, the host trees can affect the quality of medicinal loranthus to a certain extent. As a TCM, medicinal loranthus from different host trees might have varied pharmacological efficacy.

**Acknowledgments**

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**References**

13. Satish Rao, B.S., Sree devi, M.V., Nagashwar Rao, B.; Cytoprotective and antigenotoxic potential of mangiferin, a glucosylxanthone against cadmium chloride induced toxicity in HepG2 cells; *Food and Chemical Toxicology*, (2009); 47(3): 592–600.