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Full Length Research Paper

Analysis of 1-deoxynojirimycin component correlation between medicinal parasitic loranthus from loranthaceae and their mulberry host trees

Kefei Hu[1], Yonghua Li[2], Yukai Du[1], Benwei Su[2] and Dong Lu[3]*

[1]Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China.
[2]Academy of Traditional Medicine and Pharmacy of Qinzhou, Qinzhou 535000, People's Republic of China.
[3]Hospital of Chinese Traditional Medicine of Qinzhou, Qinzhou 535000, People's Republic of China.

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To analyze the component correlation of 1-deoxynojirimycin (DNJ) between medicinal parasitic loranthus from loranthaceae and its mulberry host trees as well as the effects of the host trees on the quality of parasitic loranthus. DNJ content of medicinal parasitic loranthus plants and their mulberry or non-mulberry host trees was determined by Reversed-phase High performance liquid chromatography (RP-HPLC). DNJ in samples were extracted with 0.05 mol·L^{-1} Hydrochloric acid (HCl) solution, derivatized with 9-fluorenylmethyl chloroformat, analyzed by HPLC equipped with fluorescence detector, and separated in an Agilent C_{18} column (250 × 4.6 mm, 5 µm) at 30°C. The mobile phase consisted of acetonitrile: 0.1% aqueous acetic acid (51:49, V/V) with a flow rate of 1.0 mL·min^{-1}. The fluorescence detector was operated at λ_{EX}=254 nm and λ_{EM}=322 nm. The calibration curve was linear over the concentration range of 0.0372 to 37.2 µg·mL^{-1}, r=0.9999. The average recovery was 96.42%. DNJ contents were 0.76 to 3.58 mg·g^{-1} in the mulberry tree and 0.40 to 1.72 mg·g^{-1} in parasitic loranthus parasitized mulberry host trees, respectively. The relative DNJ content of parasitic loranthus plants parasitized on mulberry tree reached as high as 33.1 to 106.2% of that in their host trees. DNJ could not be determined in parasitic loranthus parasitized non-mulberry host trees. Characteristic components in host trees could be delivered to the phytoparasitic species. Host trees affect the quality of medicinal parasitic loranthus to a certain extent.

Key words: Parasitic loranthus, mulberry, 1-deoxynojirimycin, reversed-phase high performance liquid chromatography (RP-HPLC), fluorescence detection.

INTRODUCTION

The branches and leaves of parasitic loranthus from Loranthaceae, known as *Sang Ji Sheng* in China, is an ingredient in traditional Chinese medicine used for liver and kidney reinforcement, tendon and bone strengthening, relief of rheumatic conditions, and prevention of abortion. According to the Chinese Pharmacopoeia (National Pharmacopoeia Committee, 2010), the official species of parasitic loranthus is *Taxillus chinensis* Danser (DC). However, numerous other parasitic loranthus in folk medicine are also derived from Loranthaceae, such as *Taxillus sutchuenensis* (Lecomte) DC, *Scurrula parasitica* L., and *Macrosolen tricolor* (Lecomte) DC (Gong et al., 1996), among others. Given that loranthus is a semi-parasitic plant and that its host trees comprise a complex variation in nature (Zhu et al., 2010), the host trees play an important role in affecting the quality of medicinal parasitic loranthus (Li et al., 2009). For example, *S. parasitica* L. parasitized on a host tree from Apocynaceae has a cardiotonic effect similar to its host (Zhou et al., 2009). The authors' previous study shows that cardiac glycoside content in *T. chinensis* DC parasitized on *Nerium indicum* could reach as high as

*Corresponding authors. E-mail: qzszyyld@sina.com. Tel: +86 7772878443. Fax: +86777 2878678.

Abbreviations: DNJ, Deoxynojirimycin; **RP-HPLC,** reversed-phase High performance liquid chromatography; **HCl,** hydrochloric acid; **DC,** danser; **FMOC-CL,** 9-fluorenylmethyl chloroformat; **LOD,** limits of detection; **LOQ,** limit of quantitation; **RSD,** relative standard deviation.

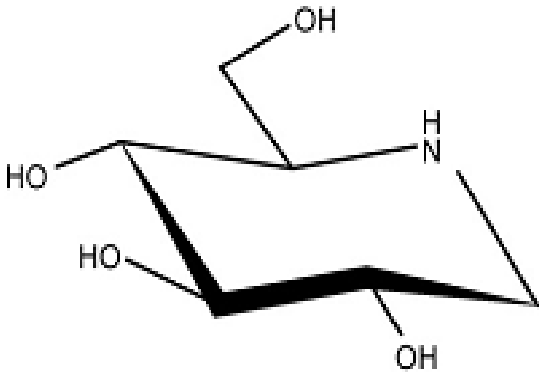


Figure 1. Chemical structure of DNJ.

84% of that in its host tree (Li et al., 2010).

The anti-diabetic activities of crude methanol extracts of *Loranthus micranthus* (Linn.) parasitized on five different host trees are shown to be different (Osadebe et al., 2004). The compositions of mistletoe from Viscaceae are host tree-dependent (Fukunaga et al., 1989; Schaller et al., 1998, 2000). Although, there is no restriction on the host trees of parasitic loranthus in Chinese pharmacopoeia, the mulberry tree has been regarded since ancient times as a common host tree for parasitic loranthus (Li et al., 2009). 1-deoxynojirimycin (DNJ, Figure 1) is a polyhydroxylated piperidine alkaloid used for diabetic control, rich in mulberry, and is the characteristic component of the mulberry tree (Kazuhisa et al., 2008; Nitra et al., 2007; Kimura et al., 2004; Kim et al., 2003). Aside from the measurement of the actual DNJ content in a host tree, whether the parasitic loranthus parasitized on a mulberry host tree contains DNJ has not been determined. In the present study, three species of parasitic loranthus and their mulberry host trees were collected to determine the DNJ contents by RP-HPLC. A species of parasitic loranthus parasitized on non-mulberry and its host tree were used as reference substances.

MATERIALS AND METHODS

Materials and reagents

DNJ (98% pure) and 9-fluorenylmethyl chloroformat (FMOC-CL, 99% pure) were purchased from Sigma-Aldrich (USA). Acetonitrile (HPLC grade) was purchased from Fisher (USA). Ultrapure water was used. All other materials and solvents were of analytical grade. Three species of parasitic loranthus samples parasitized on a mulberry tree and one species of parasitic loranthus sample parasitized on non-mulberry tree were collected from Guangxi Province in July 2010. The parasitic loranthus plants were identified by Prof. Huaxing Qiu (South China Botanical Garden, Chinese Academy of Sciences) to be *T. chinensis* DC *S. parasitica* L., and *M. tricolor* (Lecomte) DC The host trees of these parasitic plants were identified as *Morus atropurpurea* Roxb and *Nerium indicum* Mill, respectively. All samples were washed and dried. Branches and leaves were separately smashed and sieved (60 mesh) to powder for use.

Instruments and equipment

Instruments and equipment used included HPLC system (Waters Company, Type 2695), fluorescence detector (Waters Company, Type 2475), Agilent TC-C_{18} (250 × 4.6 mm, 5 μm, Agilent Company), chromatography data processing workstation, vortex mixing instrument, high-speed freezing centrifuge, adjustable microscale liquid removal instrument, and nylon syringe filter (0.45 μm).

High performance liquid chromatography (HPLC) conditions

HPLC conditions were as follows: mobile phase, acetonitrile: aqueous 0.1% acetate, 51:49, v/v; flow rate, 1.0 mL·min^{-1}; column temperature, 30°C; and fluorescence detector, excitation wavelength of 254 nm and emission wavelength of 322 nm (Kim et al., 2003).

Extraction of deoxynojirimycin (DNJ) from samples

Two hundred milligrams of sample powder was added to 20 mL aqueous 0.05 M HCl, extracted for 30 min by ultrasonication, and centrifuged at 10000 r·min^{-1} for 10 min. The supernate was saved, and the pellet was reextracted, as previously described. The first and second supernates were pooled and diluted to a final volume of 50 mL with distilled water. The diluted extract was used for subsequent derivatization (Kim et al., 2003).

Preparation of stock solution

Accurately weighed 1.86 mg of DNJ standard substance was added to 20 mL aqueous 0.05 M HCl and diluted to a final volume of 50 mL. DNJ concentration was 37.2 mg·L^{-1}. The DNJ stock solution was kept at 4°C.

Derivatization of DNJ standard solution or sample extract

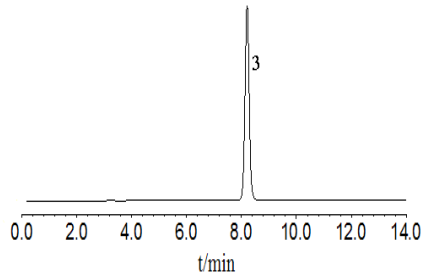
DNJ standard solution or sample extract (10 μL) was mixed with 10 μL 0.4 M potassium borate buffer (pH 8.0) in a 1.5 mL microtube. Next, 20 μL 5 mM FMOC-Cl acetonitrile solution was added and vortexed for 30 s, and then reacted in a 25°C water bath for 20 min. Then, 10 μL 0.1 M glycine (Gly) was added to terminate the reaction by quenching the remaining FMOC-Cl. The mixture was diluted with 950 μL 0.1% (v/v) aqueous acetic acid to stabilize the DNJ-FMOC and filtered through a 0.45 μm nylon syringe filter. Finally, a 10 μL aliquot of the filtrate was injected into the HPLC system to determine the DNJ content (Kim et al., 2003).

Derivatization of blank samples

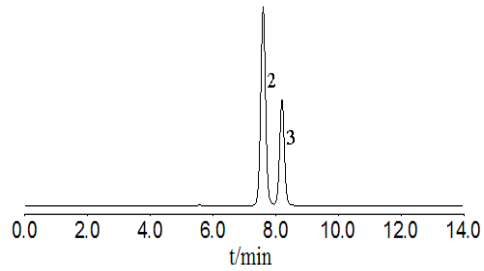
DNJ standard solution or sample extract was replaced by distilled water preceding derivatization. One of two blank groups was added to Gly. The methods used for the derivative reaction were the same as those in Section 2.6. Finally, 10 μL of the blank derivative solution was injected into the HPLC system for analysis.

Linear relationship and detection limits

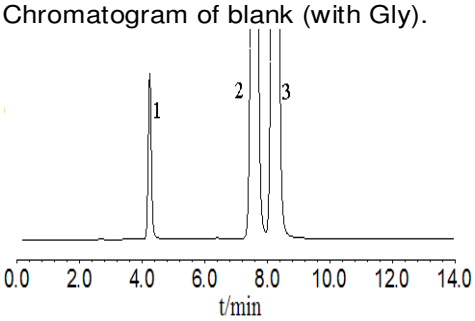
Stock solution of DNJ was diluted by aqueous 0.05 M HCl to



Chromatogram of blank (without Gly).



Chromatogram of blank (with Gly).



Chromatogram of DNJ standard (with Gly and DNJ).

Figure 2. Chromatograms of the blanks and standard DNJ, 1. FMOC-DNJ; 2. FMOC-Gly; 3. FMOC-OH.

provide a series of analytical standard working concentrations of 3.72, 7.44, 14.88, 22.32, 29.76 and 37.2 mg·L^{-1} for constructing calibration curves. The methods used for the derivative reaction were the same as those in Section 2.6. Finally, 10 µL of the derivative solution of DNJ standard was injected into the HPLC system to determine peak areas. The peak areas and DNJ concentrations were analyzed by regression methods, and calibration curves were drawn to calculate the regression equation. The limits of detection (LOD) were evaluated based on a signal-to-noise ratio of 3:1 (Kim et al., 2003).

RESULTS AND DISCUSSION

Map of high performance liquid chromatography (HPLC)

The blank derivative solution or derivative solution of DNJ standard was analyzed under chromatographic conditions. The results are shown in Figure 2. The chromatograms show Peak 3 without DNJ and Gly, Peaks 2 and 3 with Gly but without DNJ, and finally, Peaks 1, 2 and 3 with DNJ and Gly. Areas of Peak 1 vary due to the differen concentrations of DNJ. Peak 1 corresponds to FMOC-DNJ, Peak 2 corresponds to FMOC-Gly, whereas Peak 3 corresponds to FMOC-OH.

Linear relationship

The regression equation was Y=96482x-9250, and the correlation coefficient was r=0.9999. This figure proved that the concentrations and the corresponding peak areas exhibit good linearity with DNJ concentration in the range of 0.0372–37.2 µg·mL^{-1}. The limit of quantitation (LOQ) was achieved as the lowest point on the standard curve, 0.0372 µg·mL^{-1} for DNJ with relative standard deviation (RSD) of 0.62% (n=5) (He et al., 2011). LOD is defined as the lowest DNJ concentration resulting in a signal-to-noise ratio of 3:1. LOD of the sample analyte was 0.01 µg·mL^{-1} for DNJ.

Precision experiment

Derivatized extracts of the same sample (10 µL) were injected seven times into the HPLC system to determine the DNJ content. RSD was 0.69% (n=7), proving the good precision of the equipment.

Stability experiment

Derivatized extracts of the same sample (10 µL) were injected into the HPLC system to determine the DNJ content at 0, 4, 8, 16, 24, 48 and 72 h. RSD was 1.22% (n=7), showing that DNJ was stable at 72 h.

Reproducibility experiment

Five samples were accurately weighed. DNJ content was determined after extraction and derivative reaction. RSD was 1.49% (n=5), illustrating that the present method had good reproducibility.

Average recovery rate

After the DNJ content had been determined, five samples were accurately weighed, and then added to the DNJ standard. DNJ content was then determined by extraction and derivative reaction. Average recovery rate was 96.42%, and RSD was 0.97% (Table 1), proving that the present method had a high recovery rate.

DNJ determination in samples

Derivatized extracts (10 µL) of each sample were injected into the HPLC system to determine the DNJ content.

Table 1. Average DNJ recovery rate.

Quality of samples (g)	Contents of DNJ (mg)	Amount of added standards (mg)	Measured value (mg)	Recovery rate (%)	The average recovery rate (%)	RSD (%)
0.1006	0.1316	0.1488	0.2739	95.6343	96.42	0.97
0.1009	0.1320	0.1488	0.2743	95.6537		
0.1012	0.1324	0.1488	0.2782	98.0291		
0.1008	0.1318	0.1488	0.2745	95.8620		
0.1009	0.1320	0.1488	0.2762	96.9197		

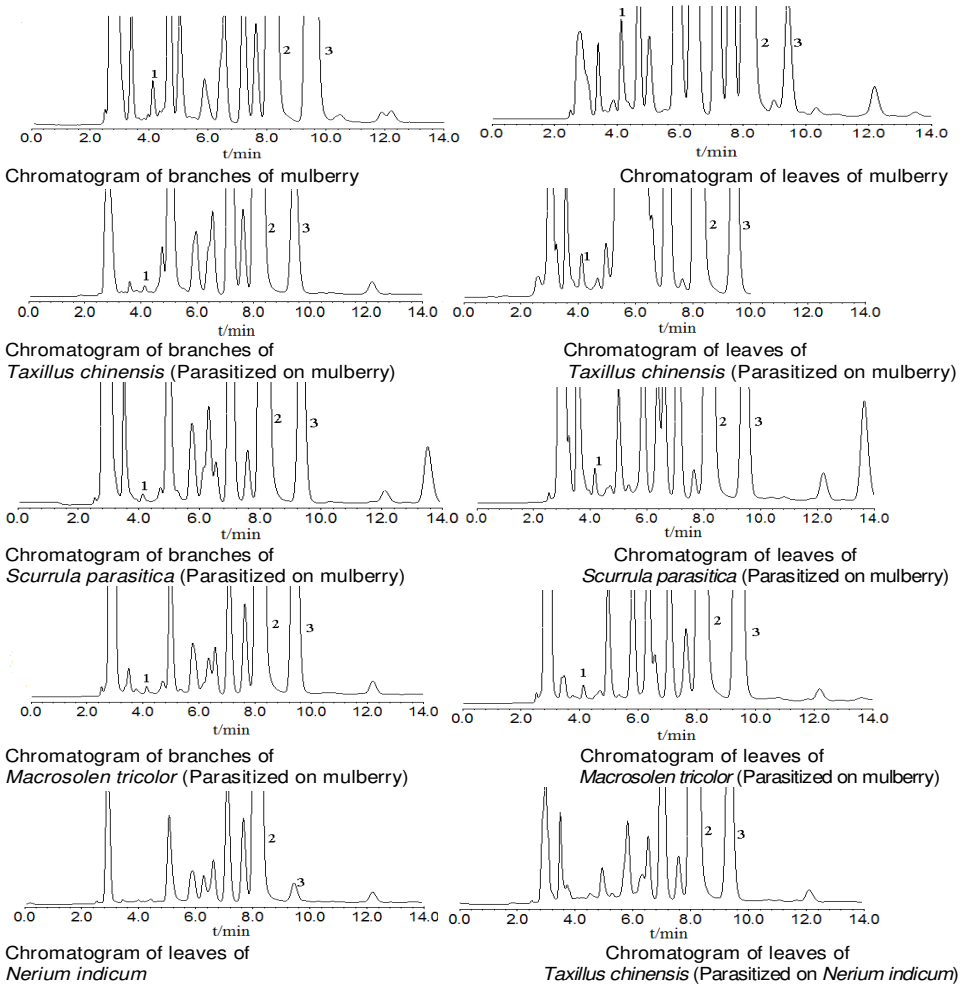


Figure 3. Chromatograms of parasitic loranthus plants and their hosts 1.FMOC-DNJ; 2.FMOC-Gly; 3.FMOC-OH.

Chromatograms of each sample are shown in Figure 3, and the results are listed in Table 2.

Characteristic component of the mulberry host tree

DNJ was found in the mulberry tree and its parasitic loranthus, whereas it was undetected in *N. indicum* Mill and its parasitic loranthus plant. The results of the experiment and analysis show that DNJ is the characteristic component of mulberry host tree. The DNJ contents in the mulberry tree were also consistent with those in the literature (Kazuhisa et al., 2008; Nitra et al., 2007; Kimura et al., 2004; Kim et al., 2003).

Table 2. DNJ determination in parasitic loranthus plants and their hosts.

Samples no.	Samples	Samples weight (g)	Retention time (min)	Contents of DNJ ($mg \cdot g^{-1}$)	Relative contents(%)[a]
1	Branches of mulberry	0.2027	4.121	1.39	
	Leaves of mulberry	0.2022	4.131	3.58	
	Branches of *Taxillus chinensis*	0.2007	4.122	0.46	33.1
	Leaves of *Taxillus chinensis*	0.2026	4.116	1.72	48.0
2	Branches of mulberry	0.2017	4.129	0.84	
	Leaves of mulberry	0.2028	4.132	1.15	
	Branches of *Scurrula parasitica*	0.2023	4.120	0.43	51.6
	Leaves of *Scurrula parasitica*	0.2021	4.121	1.22	106.2
3	Branches of mulberry	0.2029	4.122	0.76	
	Leaves of mulberry	0.2021	4.116	2.19	
	Branches of *Macrosolen tricolor*	0.2022	4.125	0.40	52.8
	Leaves of *Macrosolen tricolor*	0.2021	4.126	0.73	33.3
4	Leaves of *Nerium indicum*	0.2031	/	/	
	Leaves of *Taxillus chinensis*	0.2038	/	/	/

[a] Relative content(%) = (content of DNJ in parasitic loranthus)/(content of DNJ in the same part of it host tree)×100%.

DNJ content in the branches and leaves of parasitic loranthus

DNJ contents were different in the various medicinal parts of parasitic loranthus parasitized on a mulberry tree. DNJ contents in leaves are higher than those in branches, regardless of the species of the parasitic loranthus plant. The mechanism whereby loranthus accumulates the characteristic component of its host tree requires further exploration.

Effects of host tree on the quality of parasitic loranthus

To date, few studies have reported on the effects of host trees on the quality of parasitic loranthus (Gong et al., 1996; Zhou et al., 2009; Li et al., 2009). The results of the present study show that *T. chinensis* DC *S. parasitica* L, and *M. tricolor* (Lecomte) DC have the DNJ characteristic component of their host trees. The relative DNJ content of parasitic loranthus plants parasitized on mulberry tree reach as high as 33.1 to 106.2% of that in their host trees (Table 2). This condition may due to their parasitic relationship, implying that host trees are able to deliver certain characteristic components to the phytoparasitic species in some way, thereby affecting the quality of parasitic loranthus to some extent. Therefore, the quality of parasitic loranthus should be controlled through managing and regulating its host trees. The mechanism by which the characteristic components of host trees are delivered to their parasitic loranthus plants and whether there is variation in the clinical effects of parasitic loranthus parasitized on mulberry and non-mulberry deserve further study.

Relative DNJ contents between parasitic loranthus plants and their host trees

The relative DNJ contents between parasitic loranthus plants and their host trees are listed in Table 2. Findings show that DNJ contents are dissimilar in the different parasitic loranthus plants and different parts of the plant. The minimum relative DNJ content between parasitic loranthus plants and their host trees was 33.1%, whereas the maximum value was 106.2% (Table 2).

Conclusion

The relative DNJ content of parasitic loranthus plants parasitized on a mulberry tree could reach as high as 33.1 to 106.2% of that in their host trees. Given that DNJ is a characteristic component of a mulberry tree, the methods established in the present study could be used to control the quality of parasitic loranthus parasitized on a mulberry tree as well as to distinguish these plants from those on non-mulberry trees.

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REFERENCES

Fukunaga T, Kajikawa I, Nishiya K, Takeya K, Itokawa H (1989). Studies on the constituents of the Japanese mistletoe, *Viscum albumL. var. coloratum Ohwi* grown on different host trees. Chem. Pharm. Bull., 37: 130-1303.

Gong ZN, Wang ZT, Xu LS, Xu GJ, Wu JR (1996). Studies on medicinal plants of Loranthaceae in China. Chin. Wild Plant Res., 1: 11-15.

He F, Dou DQ, Sun Y, Kang TG (2011). Determination of Arctiin in rat plasma by HPLC method and its application to pharmacokinetic studies. J. Med. Plants Res., 4: 549-557.

Kim JW, Kim SU, Lee HS, Kim I, Ahn MY, Ryu KS (2003). Determination of 1-deoxynojirimycin in *Morus alba* L. leaves by derivatization with 9-flurenylmethyl chloroformate followed by reversed-phase high-performance liquid chromatography. J. Chromatogr. A, 1002: 93-99.

Kimura T, Nakagawa K, Saito Y, Yamagishi K, Suzuki M, Yamaki K, Shinmoto H, Miyazawa T (2004). Determination of 1-deoxynojirimycin in mulberry leaves using hydrophilic interaction chromatography with evaporative light scattering detection. J. Agric. Food Chem., 52: 1415-1418.

Li YH, Lu D, Zhu KX, Pei HH, Zhao MH, Ruan JL (2010). Interrelated study on the contents of cardiac glycosides between HerbaTaxilli and its host-plants from Apocynaceae. Lishizhen Med. Mater. Med. Res., 6: 1397-1398.

Li YH, Ruan JL, Chen SL, Lu D, Zhu KX, Zhao MH, Pei HH (2009). Study on medicinal plants of Loranthaceae resources from China. World Sci. Technol., 5: 665-669.

National Pharmacopoeia Committee (2010). Pharmacopoeia of the People's Republic of China. Chinese Medical Science and Technology Press, Beijing, pp. 280-281.

Osadebe PO, Okide GB, Akabogu IC (2004). Study on anti-diabetic activities of crude methanolic extracts of *Loranthus micranthus* (Linn.) sourced from five different host trees. J. Ethnopharm., 95: 133-138.

Schaller G, Urech K, Grazi G, Giannattasio M (1998). Viscotoxine composition of the three European subspecies of *Viscum album*. Planta Med., 64: 677-678.

Schaller G, Urech K, Grazi G (2000). Viscotoxins in the three subspecies of Viscum album L. Mistilteinn, 1: 32-40.

Zhou F, Li AY, Liao YK, Luo LP (2008). Comparative study on the cardiotonic effect between *Taxillus chinensis* (DC.) and *Scurula parasitica*. Lishizhen Med. Mater. Med. Res., 9: 2236-2237.

Zhu KX, Lu D, Pei HH, Zhao MH, Li YH (2010). Study on the distribution in Guangxi and host status of Loranthaceae. Guangxi J. Trad. Chin. Med., 2: 59-61.