Journal of Medicinal Plants Research Vol. 6(12), pp. 2474-2477, 30 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1735
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Study on salicin content correlation between taxilli herba and their willow host plants

Dong Lu[1], Benwei Su[1], Yonghua Li[2]*, Kaixin Zhu[1], Hehuan Pei[1], Minghui Zhao[1] and Jing Li[1]

[1]Qinzhou Hospital of Traditional Chinese Medicine, Qinzhou 535000, China.
[2]Guangxi Traditional Chinese Medicine University, Nanning, 530001, China.

Accepted 20 February, 2012

This paper aims to study the correlation of salicin content between taxilli herba and its host plants, willows. The salicin content of the taxilli herba parasitizing in willows and mulberries was determined by the method of reversed-phase high performance liquid chromatography (RP-HPLC). The salicin sample was ultrasonically extracted in methanol solution in the chromatographic conditions of an Ultimate®XB-C_{18} column (250 mm × 4.6 mm, 5 μm) at room temperature, with a mobile phase of methanol 0.1% potassium dihydrogen phosphate (32:68, V/V), a flow rate of 1.0 ml·min^{-1}, and a detection wavelength of 269 nm. The linear range of salicin was from 2.02 to 404.80 μg·L^{-1} (r = 1.0000), and the average recovery rate was 97.76%. The salicin content of willow host trees and of the taxilli herba parasitizing in them was in the range of 0.84 to 3.29 mg·g^{-1} and of 1.80 to 10.56 mg·g^{-1}, respectively, the latter reaching 355.17% (3.55 times) as high as the former, at the very most. However, no salicin could be determined in mulberry host trees and the taxilli herba parasitizing in them. Salicin characteristic components in willow host trees could be multiplied by their taxilli herba, and host trees could affect the medicinal quality of the taxilli herba parasitizing in them possibly by delivering their characteristic components.

Key words: Taxilli herba, willow, salicin, reversed-phase high performance liquid chromatography, ultraviolet detection.

INTRODUCTION

Having antipyretic and analgesic effects, salicin can be used for the treatment of fever and diseases, like arthritis (Teruaki et al., 2002). As a natural product, it is widely found in the family salicaceae, especially in the willow branches and barks (Zhao et al., 2005). Taxilli herba is commonly used as a traditional Chinese herbal medicine. Being the most widely used taxilli herba parasitizes in mulberries from ancient times, taxilli herba parasitizing in other host plants like daimyo oaks, beeches, willows, bigcatkin willows, and maples are still being used (Li et al., 2009; Li et al., 2006). Even in the current Chinese Pharmacopoeia, the host plants of taxilli herba are not clearly defined (National Pharmacopoeia Committee, 2010). In this study, the method of RP-HPLC is adopted to determine the salicin content of the taxilli herba parasitizing in willows and its host plants, with the taxilli herba parasitizing in mulberries and its host plants as reference substances. By observing the salicin content of taxilli herba and its host plants, this study can provide some experimental evidence for the assessment of the impact of host plants on their taxilli herba's medicinal quality.

*Corresponding author. E-mail:liyonghua185@126.com. Tel: +867713137585. Fax: +867713140360.

MATERIALS AND METHODS

Materials and reagents

The reference substance of salicin was purchased from Chengdu Mansite Pharmaceutical Co., Ltd., with the batch number of A0130. Furthermore, ultrapure water was used, and all the other chemical reagents were analytically pure. The taxilli herba parasitizing in willows and in mulberries was collected from Qinzhou, Guangxi in the wild environment; and the standardized planting base of Qinzhou Institute of Traditional Chinese Medicine respectively in March 2011. The taxilli herba was identified by researcher Huaxing Qiu from South China Institute of Botany, the Chinese Academy of

Science as *Taxillus chinensis* (DC.) Danser and the host plants as *Salix babylonica* Linn. and *Morus atropurpurea* Roxb., respectively. The samples collected were washed and dried, and their branches and leaves were separated and ground to a 45-mesh powder for use.

Instruments and equipment

The instruments and equipments used included: high performance liquid chromatograph (Japanese Shimadzu Corporation, LC-20AT), UV detector (Japanese Shimadzu Corporation, SPD-20A), Ultimate®XB-C18 column (250 mm × 4.6 mm, 5 μm), ultrasonic cleaner (Kun Shan Ultrasonic Instruments Co., Ltd, KQ-300DB), analytical balance (AND GH-252), adjustable micro pipette (Thermo), and 0.45 μm microporous membrane.

Experimental methods

Preparation of the standard solution

Accurately weighed 10.12 mg of salicin reference substance was added to the methanol solution and diluted to a constant volume of 25 ml, thus forming the mother liquor of standard solution with the concentration of 404.80 $\mu g \cdot L^{-1}$, which was then kept at a 4°C refrigerator for use.

Preparation of the sample solution

Accurately weighed 0.2 g of the sample was added to 25 ml of methanol solution. Then the sample solution was ultrasonically extracted for 30 min and finally cooled. After filtration by the 0.45 μm microporous membrane, it was kept at a 4°C refrigerator for use.

RESULTS

Chromatographic conditions and system adaptability

The detection wavelength of the salicin was 269 nm (Hui and Wang., 2004); the mobile phase was methanol 0.1% potassium dihydrogen phosphate (32:68, V/V); the column temperature was room temperature; and the flow rate was 1.0 $ml \cdot min^{-1}$. The chromatograms of both the reference substance and the sample are shown in Figure 1.

Linear relationship

The mother liquor of the reference solution was diluted by methanol to form a series of salicin reference solutions with concentrations of 2.02, 10.12, 50.60, 101.20, 202.40 and 404.80 $\mu g \cdot L^{-1}$ in turn. Then, 10 μl of each salicin reference solution with the aforementioned concentrations was injected into the HPLC system to determine the peak areas, which were used for the regression analysis of the salicin concentrations. Finally, standard curves were drawn to calculate the regression equation, which was $Y = 2065.7x + 5046.2$, with a correlation coefficient (r) of 1.0000. As a result, it could be concluded that there was a good linear relationship between the concentrations and their corresponding peak areas when the salicin ranged in concentration from 2.02 to 404.80 $\mu g \cdot L^{-1}$. The limit of detection (LOD) was 1.09 $\mu g \cdot L^{-1}$ and the limit of quantification was 3.32 $\mu g \cdot L^{-1}$ (three times of the noise).

Precision testing

Accurately weighed 10 μl of the same sample solution was repeatedly injected into the HPLC system for six times to determine the salicin content, respectively. The RSD was 0.26% (n = 6), proving the good precision of the equipments.

Stability testing

The same sample solution was accurately weighed and injected seven times into the HPLC system at 0, 2, 4, 6, 8, 12 and 24 h, respectively to determine the salicin content. The RSD was 1.36% (n = 7), showing that the salicin sample solution was stable within 24 h.

Reproducibility testing

First, six portions of sample were accurately weighed. Then after being extracted and injected, they were used to determine the salicin content. The RSD was 0.9% (n = 6), indicating that the current method had a good reproducibility.

Average recovery rate testing

Five portions of the accurately weighed sample whose salicin content had been determined were added to a certain amount of salicin reference substance. After they had been extracted and injected, the Salicin content was determined again. The average recovery rate was 97.76%, and the RSD was 1.56% (Table 1), proving that the current method had a high recovery rate and met the experimental requirements.

Determination of salicin content in samples

The samples of taxilli herba and of its host plants were accurately weighed (0.2 g) and used for the preparation of the sample solution by the methods mentioned earlier. Then 10 μl of each sample was injected into the HPLC system to determine the salicin content, whose results are shown in Table 2.

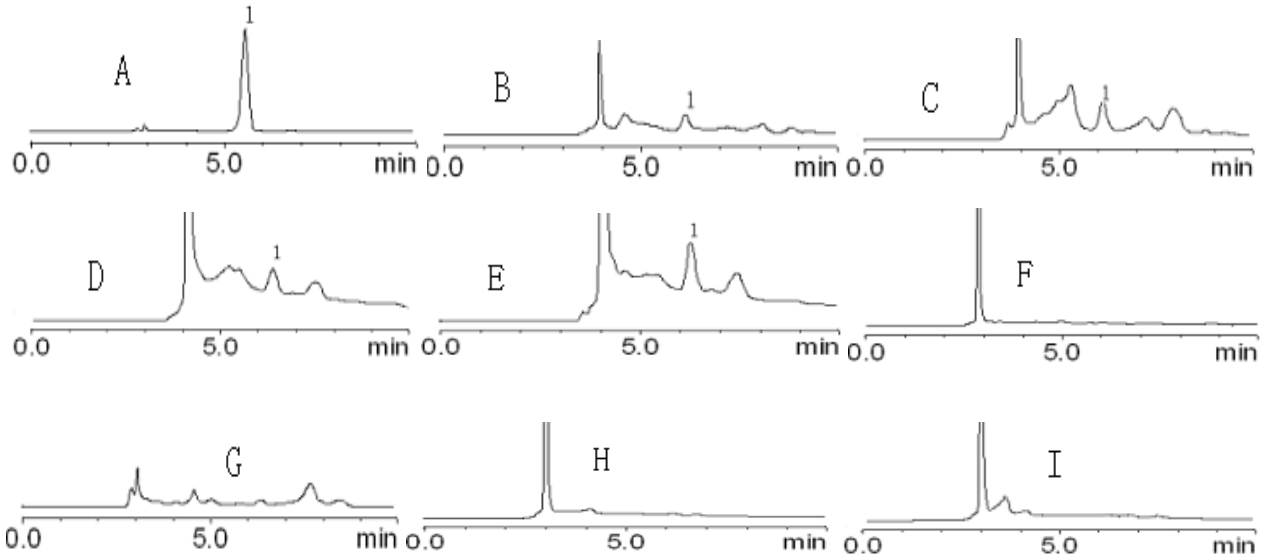

Figure 1. Chromatograms of salicin reference substance, taxilli herba, and the host plants. 1: Salicin; A: Chromatograms of salicin reference substance; B: Chromatogram of branches of willow host plants; C: Chromatogram of leaves of willow host plants; D: Chromatogram of branches of taxilli herba (in willow host plants); E: Chromatogram of leaves of taxilli herba (in willow host plants); F: Chromatogram of branches of mulberry host plants; G: Chromatogram of leaves of mulberry host plants; H: Chromatogram of branches of taxilli herba (in mulberry host plants); I: Chromatogram of leaves of taxilli herba (in mulberry host plants).

Table 1. Salicin average recovery rate.

Quality of samples (g)	Content of salicin (μg)	Amount of added standards (μg)	Measured value (μg)	Recovery rate (%)	Average recovery rate (%)	RSD (%)
0.1013	158.84	202.40	356.66	97.74	97.76	1.56
0.1025	160.72	202.40	356.16	96.56		
0.1019	159.78	202.40	354.44	96.18		
0.1006	157.74	202.40	353.08	100.00		
0.1012	158.68	202.40	357.70	98.33		

DISCUSSION

Relative salicin content between taxilli herba and their host plants

The taxilli herba medicinal materials recorded in Chinese Pharmacopoeia are the dry leaves and branches of *T. chinensis* (DC) Danser. The relative salicin content between taxilli herba and their host plants is listed in Table 2. The current experiment results of the salicin content determination show that salicin components inherent in willow host trees can be multiplied by their taxilli herba and the accumulating amount of salicin is different in different parts of the medicinal materials. The ratios of salicin content in the leaves and branches of taxilli herba medicinal materials to their corresponding parts in willow host plants are in the range of 214.29 to 355.17% (2.14 to 3.55 times).

Impact of host plants on the quality of taxilli herba

It can be seen from the determined results of the salicin content of the taxilli herba parasitizing in willows and mulberries and its host-plants, that salicin is the inherent component in willow host plants, and that taxilli herba contains salicin because of its parasitizing in willows. However, no salicin component can be found in the taxilli herba parasitizing in mulberries owing to the fact that its mulberry host plants contain no salicin. The experiment results are consistent with one of the authors' reports that the 1-deoxynojirimycin inherent in mulberry host plants could be accumulated by the taxilli herba parasitizing in them (Hu et al., 2011; Li et al., 2011). The experiment results again indicate that host plants are likely to be the key factors affecting taxilli herba medicinal materials' quality, and that host trees can affect the medicinal quality of the taxilli herba parasitizing in them by

Table 2. Determination of salicin content in taxilli herba medicinal materials and their host plants.

Samples no.	Samples	Samples weight (g)	Retention time (min)	Peak areas	Contents of salicin (mg·g^{-1})	Relative contents (%)[a]
1	Branches of Willow	0.2036	6.044	16759	0.84	
	Leaves of Willow	0.2048	5.968	17596	0.87	
	Branches of *Taxillus chinensis*	0.2016	6.003	35595	1.80	214.29
	Leaves of *Taxillus chinensis*	0.2020	6.007	61376	3.09	355.17
2	Branches of Willow	0.2017	6.016	20990	1.06	
	Leaves of Willow	0.2012	5.936	65024	3.29	
	Branches of *Taxillus chinensis*	0.2039	6.023	60481	3.02	284.91
	Leaves of *Taxillus chinensis*	0.2011	5.933	208725	10.56	320.97
3	Branches of mulberry	0.2031	/	/	/	
	Leaves of mulberry	0.2023	/	/	/	
	Branches of *Taxillus chinensis*	0.2038	/	/	/	
	Leaves of *Taxillus chinensis*	0.2048	/	/	/	

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

delivering their inherent components.

Quality control of taxilli herba medicinal materials

Given the fact that salicin is an inherent component in willow host plants, and that taxilli herba contains salicin owing to its parasitizing in willows, the method established in the current study could be used to control the medicinal materials quality of the taxilli herba parasitizing in its willow host plants, as well as to distinguish the medicinal materials of the taxilli herba parasitizing in its willow host plants from those of non-willow trees.

Conclusion

Salicin characteristic components in willow host trees could be multiplied by their taxilli herba, and host trees could affect the medicinal quality of the taxilli herba parasitizing in them possibly by delivering their characteristic components.

ACKNOWLEDGEMENTS

This research was supported by both the National Natural Science Foundation Project (81173537) and the Natural Science Foundation Project of Guangxi (2010GXNSFA013264).

REFERENCES

Hu KF, Li YH, Du YK, Su BW, Lu D (2011). Analysis of 1-deoxynojirimycin component correlation between medicinal parasitic loranthus from Loranthaceae and their mulberry host trees. J. Med Plants Res., 5(17): 4326-4331.

Hui YH, Wang RC (2004). Determination of Salicin content in Salix alba L. extract by RP-HPLC method. Chin. Trad. Herbal Drugs, 35(5): 524-525.

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 Tech: Mod. Trad. Chin. Med., 11(5): 665-669.

Li YH, Lu D, Zhao MH, Zhu KX (2006). Research on the developments and applications for medicinal plants of loranthaceae in Guangxi . Guangxi J. Trad. Chin. Med., 28(11): 1695-1698.

National Pharmacopoeia Committee (2010). Pharmacopoeia of the People's Republic of China. Chin. Med. Sci. Technol. Press, Beijing, pp. 280-281.

Li YH, Bu BW, Zhang XJ, Zhu KX, Pei HH, Zhao MH, Lu D(2011). Correlation of DNJ between Taxilli Herba and its host-plants. China J. Chin. Mater. Med., 36(15): 2102-2106.

Teruaki A, Tetsuro Y, Kyoich K, Masao H (2002). Evaluation of salicin as an Antipyretic Prodrug that does not cause Gastric Injury. Planta Med., 68(8): 714-718.

Zhao HM, Xu H, Tang W (2005). Determination of Salicin in Extract of Widdow Bark by RP-HPCL Method. Chin. Wild. Plant Res., 24(2): 39-58.