

## DISTRIBUTION OF PACLITAXEL AND ITS PRECURSORS IN DIFFERENT PARTS OF *TAXUS WALLICHIANA* VAR. *MAIREI*

JIAN ZAIYOU\*, XU GUIFANG AND CHEN HONGZHI

*Henan Institute of Science and Technology, Xinxiang, China, 453003; Henan Collaborative Innovation Center in Modern Biological Breeding, China, 453003*

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### Abstract

To understand the distributions of paclitaxel and its precursors in the different parts of yew this study was conducted. The contents of paclitaxel and its precursors in the different parts of *Taxus wallichiana* var. *mairei* were determined with RP-HPLC. The contents of paclitaxel and its precursors in yew root are higher than those in other parts. The content of paclitaxel in the bark of *Taxus wallichiana* var. *mairei* is higher than that in leaf. But there was very little paclitaxel in the xylem and tender stem of *Taxus wallichiana* var. *mairei*. There were considerable paclitaxel precursors in the leaf of *Taxus wallichiana* var. *mairei*, but low contents in 2 - 3 years stem. The root of *Taxus wallichiana* var. *mairei* can be utilized also. It is reasonable measures of satisfying the demand of people on paclitaxel and preventing people cutting yew that artificially synthesize paclitaxel with the precursors extracted from yew leaf.

### Introduction

As a naturally occurring chemical component, paclitaxel is widely used in medicine for its high anticancer activity. Paclitaxel was found for the first time from the bark of yew. But the content of paclitaxel in yew bark is of very low level (Wani *et al.* 1971). Lots of yew have been seriously damaged even destroyed to extract paclitaxel in recent years. Baccatin III and 10-deacetyl baccatin III are the precursors of paclitaxel in paclitaxel synthesis. There should be paclitaxel and its precursors in all parts of yew. The procedure to artificially synthesize paclitaxel artificially from baccatin III is relatively easy (Jennewein *et al.* 2001, Walker *et al.* 2002, Croteau *et al.* 2006 and Cusido *et al.* 2007). It can satisfy the demand of people on paclitaxel and prevent people cutting yew that artificially synthesize paclitaxel with the precursors extracted from yew. Thus, the yew resources can be effectively protected. Therefore, the contents of paclitaxel and its precursors in the root, bark, xylem, tender stem and leaf of *Taxus wallichiana* var. *mairei* were determined and analyzed to reasonably utilize yew resources.

### Materials and Methods

Shimadzu HPLC-2010 instrument, electronic analytic balance (precision: 0.0001), ultrasonicator. Methanol (AR). ethanol (AR). Standard 10-deacetyl baccatin III, baccatin III and paclitaxel (99.5%) were purchased from Sigma company.

The root, bark, xylem, tender stem and leaf of *T. wallichiana* var. *mairei* were collected in the Taihang mountain in Henan province of China in May 2017. These materials were randomly collected from above 10 plants of *T. wallichiana* var. *mairei*. The collected samples were dried to get constant weight at 40°C.

The materials were crushed and sieved with 80 meshes sieve. Each material was weighed for 2 g and extracted with 30 ml mixture of chloroform/methanol (1 : 1, v/v) in ultrasonic bath for 30 min. Then the mixture was filtered with filter paper. The residue was extracted with the same solvent

\*Author for correspondence: <jian19732004@126.com>.

and filtered once again. The filtrate was merged and evaporated to dryness under reduced pressure at 40°C in the rotary vacuum evaporator. The dry extract was dissolved with 30 ml chloroform and then the solution was mixed with 30 ml water to extract paclitaxel and its precursors. The organic phase was collected and evaporated to dryness under reduced pressure at 40°C in the rotary vacuum evaporator again. The dry residue was dissolved with 5 ml methanol and then filtered with 0.22 µm filter membrane.

Standard paclitaxel, baccatin III and 10-deacetyl baccatin III solutions were respectively, prepared at 0.0005, 0.001, 0.004, 0.01, 0.02 and 0.05 mg/ml.

The Shimadzu C18 reverse-phase column (5 µm, 250 × 4.6 mm) was used as HPLC column. The gradient mobile phase consists of acetonitrile and water. The content (v/v) of acetonitrile in the gradient mobile phase varied from 27 to 30% in 0 - 15 min, 30 to 37% in 15 - 30 min, 37 to 42 % in 30 - 40 min, 42 to 47 % in 40 - 60 min and 47 to 48% in 60 - 72 min. The flow rate of mobile phase was 0.8 ml/min. The temperature in HPLC column was 35°C. The volume of extract injected was 10 µl. A variable wavelength recorder was set at 228 nm to detect ingredients eluted from the column.

Those standard solutions and these prepared extracts were respectively, analyzed according to the above HPLC method. Chromatography peak areas of paclitaxel and its precursors in each chromatogram were respectively, recorded.

The contents of paclitaxel and its precursors in extracts were analyzed according to their chromatography peak areas and the standard curves (relating these peak areas to their corresponding contents) with SPSS (Statistical Product and Service Solutions).

## Results and Discussion

The HPLC chromatograms of standard paclitaxel and its precursors are presented in Fig. 1. These standard curves of paclitaxel and its precursors were set up according to their contents and their corresponding peak areas (Table 1).

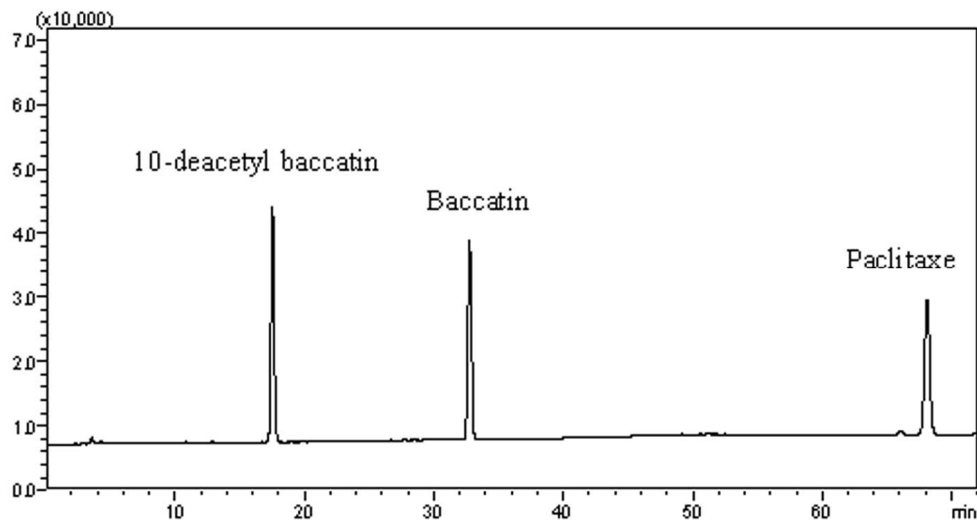


Fig. 1. HPLC chromatograms of standard paclitaxel and its precursors.

**Table 1. Peak area and contents of standard paclitaxel and its precursors.**

10-deacetyl baccatin III (Retention time 17.61 min)		Baccatin III (Retention time 32.861 min)		Paclitaxel (Retention time 68.201 min)	
Conc. (mg/ml)	Peak area	Conc. (mg/ml)	Peak area	Conc. (mg/ml)	Peak area
0.0005	18751	0.0005	11766	0.0005	27185
0.001	33228	0.001	27748	0.001	39277
0.004	156183	0.004	122540	0.004	119104
0.01	302621	0.01	261996	0.01	292557
0.02	601763	0.02	581179	0.02	570140
0.05	1482652	0.05	1461443	0.05	1411213

These standard curves of paclitaxel, baccatin III and 10-deacetyl baccatin III are respectively,  $y = 3 \times 10^7 x + 10895$  ( $R^2 = 0.9999$ ),  $y = 3 \times 10^7 x - 6273.3$  ( $R^2 = 0.9995$ ) and  $y = 3 \times 10^7 x + 13639$  ( $R^2 = 0.9995$ ) (x: Concentration and y: Peak area).

The peaks of paclitaxel and its precursors in extract chromatograms were identified according to their retention time in HPLC (Fig. 2). The concentrations of paclitaxel and its precursors in extracts were respectively, analyzed according to their peak areas and corresponding standard curves (Table 2). Then the contents of these chemical compositions in yew materials were analyzed according the method of preparation extract.

It can be seen that there are obvious difference between the contents of paclitaxel and its precursors in different parts of *Taxus wallichiana* var. *mairi* (Table 2). The results of variance analysis on these contents of paclitaxel and its precursors showed that the difference is significant. The multiple comparisons of these chemical composition contents are presented in Table 3.

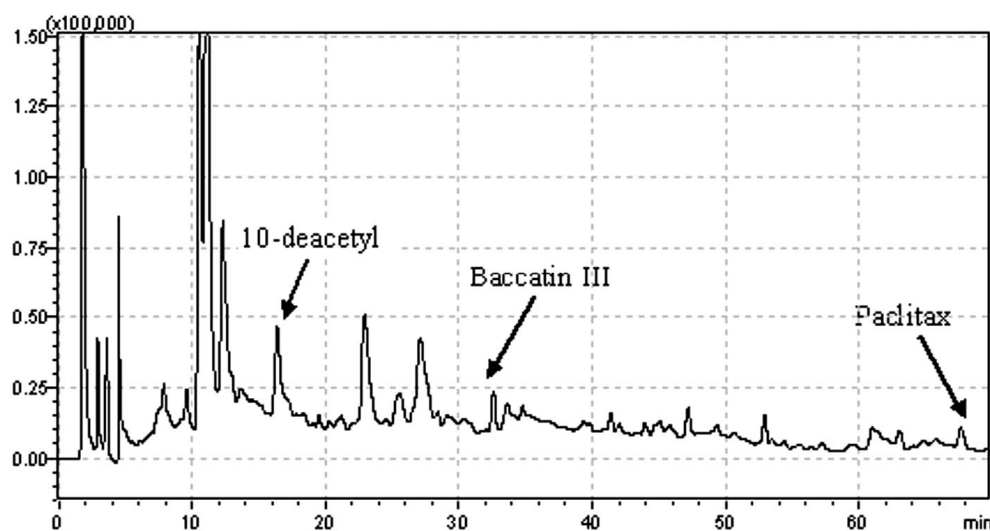


Fig. 2. HPLC chromatograms of yew materials.

**Table 2 Contents of paclitaxel and its precursors in yew materials.**

Part	10-deacetyl baccatin III			Baccatin III			Paclitaxel		
	Peak area	Conc. (mg/ml) in extracts	Content (mg/g)	Peak area	Conc. (mg/ml) in extracts	Content (mg/g)	Peak area	Conc. (mg/ml) in extracts	Content (mg/g)
2 - 3 y stem	163778	0.00511	0.0128	41356	0.00163	0.00408	54629	0.00156	0.00391
	147947	0.00457	0.0114	41562	0.00164	0.00410	45327	0.00123	0.00308
	141538	0.00436	0.0109	42765	0.00168	0.00420	47281	0.00130	0.00325
Over 5 y bark	264216	0.00853	0.0213	40443	0.00160	0.00400	378093	0.01311	0.03278
	274185	0.00887	0.0222	45375	0.00177	0.00442	352682	0.01221	0.03052
	266683	0.00861	0.0215	45036	0.00176	0.00440	359998	0.01247	0.03117
Over 5 y root	1081243	0.03631	0.0908	610930	0.02107	0.05267	770115	0.02711	0.06778
	1059473	0.03557	0.0889	657916	0.02267	0.05668	668058	0.02347	0.05867
	1148856	0.03861	0.0965	791001	0.02721	0.06803	716548	0.02520	0.06300
Over 5 y xylem	256502	0.00827	0.0207	246209	0.00862	0.02156	20574	0.00035	0.00087
	246837	0.00794	0.0198	251404	0.00880	0.02200	17760	0.00025	0.00061
	217361	0.00693	0.0173	294483	0.01027	0.02568	16610	0.00020	0.00051
1 y tender stem	201544	0.00640	0.0160	398244	0.01381	0.03453	57328	0.00166	0.00415
	209516	0.00667	0.0167	375515	0.01304	0.03259	58614	0.00170	0.00426
	149449	0.00463	0.0116	381545	0.01324	0.03310	52511	0.00149	0.00372
Leaf	343870	0.01124	0.0281	282942	0.00988	0.02469	123521	0.00402	0.01006
	367564	0.01204	0.0301	305667	0.01065	0.02663	105819	0.00339	0.00848
	325627	0.01062	0.0265	224882	0.00790	0.01974	111024	0.00358	0.00894

**Table 3. Multiple comparisons of chemical components contents in all yew parts.**

Parts	10-deacetyl baccatin III		Baccatin III		Paclitaxel	
	Mean contents comparison* (mg/g)		Mean contents comparison* (mg/g)		Mean contents comparison* (mg/g)	
Over 5 y root	0.09207 <sup>a</sup>		Over 5 y root	0.05913 <sup>a</sup>	Over 5 y root	0.06315 <sup>a</sup>
Leaf	0.02823 <sup>b</sup>		1 y tender stem	0.03341 <sup>b</sup>	Over 5 y bark	0.03149 <sup>b</sup>
Over 5 y bark	0.02167 <sup>c</sup>		Leaf	0.02369 <sup>bc</sup>	Leaf	0.009157 <sup>c</sup>
Over 5 y xylem	0.01927 <sup>cd</sup>		Over 5 y xylem	0.02308 <sup>c</sup>	1 y tender stem	0.004041 <sup>d</sup>
1 y tender stem	0.01477 <sup>de</sup>		Over 5 y bark	0.004273 <sup>d</sup>	2-3 y stem	0.00341 <sup>d</sup>
2-3 y stem	0.0117 <sup>e</sup>		2-3 y stem	0.004127 <sup>d</sup>	Over 5 y xylem	0.000663 <sup>d</sup>

The mean difference is significant at the  $p < 0.05$  level. The different letters indicate obvious difference between these means, The same letters indicate there is not obvious difference between these means.

The result of multiple comparisons showed that the contents of paclitaxel and its precursors in yew root were higher than those in other parts. The content of paclitaxel in the bark of *Taxus wallichiana* var. *mairei* was higher than that of leaf. There is very little paclitaxel in the xylem and tender stem of *Taxus wallichiana* var. *mairei*. There are considerable paclitaxel precursors in the leaf of *Taxus wallichiana* var. *mairei*, but low contents in 2 - 3 years stem.

The result showed that there was considerable content of paclitaxel in yew bark is in conformity with the reports of Zhang *et al.* (2008), Kong *et al.* (2011), Li *et al.* (2011). There is very few reports about paclitaxel and its precursors in yew root. In the present study it was found that the contents of paclitaxel and its precursors in yew root were higher than those in other parts of yew. The root of *Taxus wallichiana* var. *mairei* can be utilized also. The distributions of paclitaxel and its precursors in yew help in studying the synthesise and transport of paclitaxel. Their contents of paclitaxel precursors in the leaf of *Taxus wallichiana* var. *mairei* were considerably high. Baccatin III is proximate precursor of paclitaxel (Liu *et al.* 2006). It is reasonable measures of satisfying the demand of people on paclitaxel and preventing people cutting yew that artificially synthesise paclitaxel with the precursors extracted from yew leaf.

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