

Supplementary data

Biomonitoring for traditional herbal medicinal products using DNA metabarcoding and single molecule, real-time sequencing

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Table S1 Proportions of the herbal materials in JWQHW.

Herbal material name	Proportion in the preparation
Notopterygii Rhizoma et Radix (Qianghuo)	15 %
Saposhnikoviae Radix (Fangfeng)	15 %
Atractylodis Rhizoma (Cangzhu)	15 %
Asari Radix et Rhizoma (Xixin)	5 %
Chuanxiong Rhizoma (Chuanxiong)	10 %
Angelicae dahuricae Radix (Baizhi)	10 %
Rehmanniae Radix (Dihuang)	10 %
Scutellariae Radix (Huangqin)	10 %
Glycyrrhizae Radix et Rhizoma (Gancao)	10 %

Table S2 PCR primers for the ITS2 and *psbA-trnH* regions.

Barcode	Primer	Forward (5'-3')	Reverse (5'-3')
ITS2	ZCY01	<u>TATAG</u> ATGCGATACTTGGTGTGAAT	<u>TATAG</u> GACGCTTCTCCAGACTACAAT
	ZCY02	<u>AAGTC</u> ATGCGATACTTGGTGTGAAT	<u>AAGTC</u> GACGCTTCTCCAGACTACAAT
	ZCY03	<u>CCTAG</u> ATGCGATACTTGGTGTGAAT	<u>CCTAG</u> GACGCTTCTCCAGACTACAAT
	ZCY04	<u>GGAAT</u> ATGCGATACTTGGTGTGAAT	<u>GGAAT</u> GACGCTTCTCCAGACTACAAT
	ZCY05	<u>ACACT</u> ATGCGATACTTGGTGTGAAT	<u>ACACT</u> GACGCTTCTCCAGACTACAAT
<i>psbA-trnH</i>	ZCY11	<u>GTATG</u> GTTATGCATGAACGTAATGCTC	<u>GTATG</u> CGCGCATGGTGGATTACAATCC
	ZCY12	<u>TGAAT</u> GTTATGCATGAACGTAATGCTC	<u>TGAAT</u> CGCGCATGGTGGATTACAATCC
	ZCY13	<u>TAATC</u> GTTATGCATGAACGTAATGCTC	CGCGCATGGTGGATTACAATCC
	ZCY14	<u>AAGTC</u> GTTATGCATGAACGTAATGCTC	<u>AAGTC</u> CGCGCATGGTGGATTACAATCC
	ZCY15	<u>GCGTA</u> GTTATGCATGAACGTAATGCTC	<u>GCGTA</u> CGCGCATGGTGGATTACAATCC

“ ” showed the 5 bp tag.

Table S3 Identification results of the herbal ingredients of JWQHW by ITS2 and *psbA-trnH* sequence.

Name	ITS2	<i>psbA-trnH</i>
Notopterygii Rhizoma et Radix (Qianghuo)	√	√
Saposhnikoviae Radix (Fangfeng)	√	—
Atractylodis Rhizoma (Cangzhu)	—	—
Asari Radix et Rhizoma (Xixin)	√	—
Chuanxiong Rhizoma (Chuanxiong)	√	—
Angelicae Dahuricae Radix (Baizhi)	—	—
Scutellariae Radix (Huangqin)	√	—
Rehmanniae Radix (Dihuang)	√	—
Glycyrrhizae Radix et Rhizoma (Gancao)	√	√

Table S4 The CCS results from the RS_ReadsOfInsert.1 protocol of the SMRT portal.

Job metric	Value
Read bases of insert	4,554,944 bp
Mean read length of insert	422.0 bp
Mean read quality of insert	99.8%
Mean number of passes	59.0

Table S5 Number of CCS reads of the prescribed herbal materials detected in five JWQHW samples with SMRT sequencing based on the ITS2 and *psbA-trnH* regions.

Name	JWQHW01		JWQHW02		JWQHW03		RF01		RF02	
	ITS2	<i>psbA-trnH</i>	ITS2	<i>psbA-trnH</i>	ITS2	<i>psbA-trnH</i>	ITS2	<i>psbA-trnH</i>	ITS2	<i>psbA-trnH</i>
Notopterygii Rhizoma et Radix (Qianghuo)	112	3	491		444	12	32	19	31	18
Saposhnikoviae Radix (Fangfeng)		1	14		200		107		122	
Atractylodis Rhizoma (Cangzhu)	1	6	10		8	1				
Asari Radix et Rhizoma (Xixin)	165		71		187		21		45	
Chuanxiong Rhizoma (Chuanxiong)	27	288	42	5	5	1	523	1436	568	1193
Scutellariae Radix (Huangqin)		3				1		68		68
Rehmanniae Radix (Dihuang)							2		3	
Glycyrrhizae Radix et Rhizoma (Gancao)	215	29	209	2	190	9	326	230	342	242
Angelicae Dahuricae Radix (Baizhi)										
<i>Panax ginseng</i>										9

Table S6 Number of CCS reads of the adulterant and contaminant species detected in five JWQHW samples with SMRT sequencing based on the ITS2 and *psbA-trnH* regions.

No.	Family	Genus	JWQHW01		JWQHW02		JWQHW03		RF01		RF02	
			ITS2	<i>psbA-trnH</i>	ITS2	<i>psbA-trnH</i>	ITS2	<i>psbA-trnH</i>	ITS2	<i>psbA-trnH</i>	ITS2	<i>psbA-trnH</i>
1	Aceraceae	<i>Acer</i>	3									
2	Actinidiaceae	<i>Actinidia</i>	2									
3	Alismataceae	<i>Alisma</i>					1					
4	Amaranthaceae	<i>Amaranthus</i>	1									
5	Asteraceae	<i>Artemisia</i>	1				1					
6		<i>Atractylodes</i>	27		1							
7		<i>Cirsium</i>					1					
8		<i>Saussurea</i>			1							
9		<i>Taraxacum</i>					1					
10	Caprifoliaceae	<i>Sambucus</i>			1							
11	Chenopodiaceae	<i>Chenopodium</i>		1			2					
12	Convolvulaceae	<i>Convolvulus</i>	3		1							

13		<i>Cuscuta</i>	2						
14		<i>Ipomoea</i>	4		5		3		
15	Cruciferae	<i>Isatis</i>	1						
16	Cucurbitaceae	<i>Citrullus</i>						4	
17		<i>Trichosanthes</i>						1	
18	Elaeagnaceae	<i>Hippophae</i>			2		1		
19	Ericaceae	<i>Rhododendron</i>	1		16		13		
20	Fabaceae	<i>Alhagi</i>			1				
21		<i>Cullen</i>	1						
22		<i>Hedysarum</i>			1			1	
23		<i>Lablab</i>			4				
24		<i>Phaseolus</i>			1				
25		<i>Vicia</i>	4		3		1		
26		<i>Vigna</i>						1	
27	Geraniaceae	<i>Erodium</i>	1	1					
28		<i>Geranium</i>		1	1		1		
29	Gramineae	<i>Digitaria</i>					1		
30	Juglandaceae	<i>Juglans</i>		1				1	
31	Lamiaceae	<i>Isodon</i>					1		
32		<i>Perilla</i>			1				
33		<i>Pogostemon</i>			2		1	10	
34	Loranthaceae	<i>Scurrula</i>		16				1	
35		<i>Taxillus</i>		28					
36	Oleaceae	<i>Fraxinus</i>			1				
37	Paeoniaceae	<i>Paeonia</i>	120	34	5		6	1	
38	Pinaceae	<i>Picea</i>			1				
39	Polygonaceae	<i>Rumex</i>						57	
40	Rhamnaceae	<i>Ziziphus</i>		2					
41	Rosaceae	<i>Prunus</i>			1				
42		<i>Rosea</i>					1		
43		<i>Rubus</i>	1		2				
44		<i>Sanguisorba</i>	1					3	1 2
45		<i>Spiraea</i>	1						
46	Rutaceae	<i>Zanthoxylum</i>			1				
47	Salicaceae	<i>Salix</i>		1	2			2	
48	Tiliaceae	<i>Tilia</i>	1						
49	Umbelliferae	<i>Angelica</i>		7	287		89	256	126 1 118
50		<i>Bupleurum</i>	1		4		2		
51		<i>Foeniculum</i>	3						
52		<i>Heracleum</i>			3		1		
53		<i>Ligusticum</i>	1				39	1	
54		<i>Peucedanum</i>	192	12	2		6		1
55		<i>Pimpinella</i>		3			2	1	1 2
56		<i>Pleurospermum</i>					2		
57	Verbenaceae	<i>Tectona</i>							3