

**Rapidly discriminate commercial medicinal *Pulsatilla chinensis*
(Bge.) Regel from its adulterants using ITS2 barcoding and specific
PCR-RFLP assay**

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Supplementary Information

Supplementary Table S1 Information of voucher samples used in the study

Species	Family	Sample ID	Voucher number	Collected part	Collected place	GenBank accession
<i>Pulsatilla chinensis</i>	Ranunculaceae	B1	PS0899B1	leaf	Nanyang, Henan	KR611721
<i>Pu. chinensis</i>	Ranunculaceae	B2	PS0899B2	leaf	Nanyang, Henan	KR611722
<i>Pu. chinensis</i>	Ranunculaceae	B3	PS0899B3	leaf	Nanyang, Henan	KR611723
<i>Pu. chinensis</i>	Ranunculaceae	B4	PS0899B4	leaf	Nanyang, Henan	KR611724
<i>Pu. chinensis</i>	Ranunculaceae	B5	PS0899B5	leaf	Nanyang, Henan	KR611725
<i>Pu. chinensis</i>	Ranunculaceae	B6	PS0899B6	leaf	Nanyang, Henan	KR611726
<i>Pu. chinensis</i>	Ranunculaceae	B7	PS0899B7	root	Nanyang, Henan	KR611727
<i>Pu. chinensis</i>	Ranunculaceae	B8	PS0899B8	root	Nanyang, Henan	KR611728
<i>Pu. chinensis</i>	Ranunculaceae	B9	PS0899B9	root	Nanyang, Henan	KR611729
<i>Pu. chinensis</i>	Ranunculaceae	B10	PS0899B10	root	Nanyang, Henan	KR611730
<i>Pu. chinensis</i>	Ranunculaceae	B11	PS0899B11	leaf	Xi'an, Shaanxi	KR611731
<i>Pu. chinensis</i>	Ranunculaceae	B12	PS0899B12	leaf	Xi'an, Shaanxi	KR611732
<i>Pu. chinensis</i>	Ranunculaceae	B13	PS0899B13	leaf	Xi'an, Shaanxi	KR611733
<i>Pu. chinensis</i>	Ranunculaceae	B14	PS0899B14	leaf	Shengnongjia, Hubei	KR611734
<i>Pu. chinensis</i>	Ranunculaceae	B15	PS0899B15	leaf	Shengnongjia, Hubei	KR611735
<i>Pu. chinensis</i>	Ranunculaceae	B16	PS0899B16	leaf	Shengnongjia, Hubei	KR611736
<i>Pu. chinensis</i>	Ranunculaceae	B17	PS0899B17	leaf	Baihuashan, Beijing	KR611737
<i>Pu. chinensis</i>	Ranunculaceae	B18	PS0899B18	leaf	Baihuashan, Beijing	KR611738
<i>Pu. chinensis</i>	Ranunculaceae	B19	PS0899B19	leaf	Baihuashan, Beijing	KR611739
<i>Pu. chinensis</i>	Ranunculaceae	B20	PS0899B20	leaf	Changbaishan, Jilin	KR611740
<i>Pu. chinensis</i>	Ranunculaceae	B21	PS0899B21	leaf	Changbaishan, Jilin	KR611741
<i>Pu. chinensis</i>	Ranunculaceae	B22	PS0899B22	leaf	Changbaishan, Jilin	KR611742
<i>Pu. chinensis</i>	Ranunculaceae	B23	PS0899B23	leaf	Beijing	KR611743
<i>Pu. chinensis</i>	Ranunculaceae	B24	PS0899B24	leaf	Beijing	KR611744
<i>Pu. chinensis</i>	Ranunculaceae	B25	PS0899B25	leaf	Beijing	KR611745
<i>Pu. cernua</i>	Ranunculaceae	C1	PS3102C1	leaf	Dandong, Liaoning	KT969417
<i>Pu. cernua</i>	Ranunculaceae	C2	PS3102C2	leaf	Dandong, Liaoning	KT969418
<i>Pu. cernua</i>	Ranunculaceae	C3	PS3102C3	leaf	Dandong, Liaoning	KT969419
<i>Pu. cernua</i>	Ranunculaceae	C4	PS3102C4	leaf	Dandong, Liaoning	KT969420
<i>Pu. cernua</i>	Ranunculaceae	C5	PS3102C5	leaf	Dandong, Liaoning	KT969421
<i>Pu. cernua</i>	Ranunculaceae	C6	PS3102C6	leaf	Dandong, Liaoning	KT969422
<i>Pu. cernua</i>	Ranunculaceae	—	—	—	GenBank	HQ829820
<i>Pu. cernua</i>	Ranunculaceae	—	—	—	GenBank	JN811070
<i>Pu. cernua</i>	Ranunculaceae	—	—	—	GenBank	JN811071
<i>Pu. campanella</i>	Ranunculaceae	Z1	PS3103Z1	leaf	Wulumuqi, Xinjiang	KT969423
<i>Pu. campanella</i>	Ranunculaceae	Z2	PS3103Z2	leaf	Wulumuqi, Xinjiang	KT969424
<i>Pu. campanella</i>	Ranunculaceae	Z3	PS3103Z3	leaf	Wulumuqi, Xinjiang	KT969425
<i>Pu. campanella</i>	Ranunculaceae	Z4	PS3103Z4	leaf	Wulumuqi, Xinjiang	KT969426

<i>Pu. campanella</i>	Ranunculaceae	Z5	PS3103Z5	leaf	Wulumuqi, Xinjiang	KT969427
<i>Pu. campanella</i>	Ranunculaceae	Z6	PS3103Z6	leaf	Wulumuqi, Xinjiang	KT969428
<i>Pu. campanella</i>	Ranunculaceae	Z7	PS3103Z7	leaf	Wulumuqi, Xinjiang	KT969429
<i>Pu. campanella</i>	Ranunculaceae	Z8	PS3103Z8	leaf	Wulumuqi, Xinjiang	KT969430
<i>Pu. millefolium</i>	Ranunculaceae	X1	PS3104X1	leaf	Yunnan	KT969431
<i>Pu. tongkanensis</i>	Ranunculaceae	—	—	—	GenBank	JN811072
<i>Pu. tongkanensis</i>	Ranunculaceae	—	—	—	GenBank	JN811073
<i>Pu. tongkanensis</i>	Ranunculaceae	—	—	—	GenBank	JN811074
<i>Pu. tongkanensis</i>	Ranunculaceae	—	—	—	GenBank	JN811075
<i>Pu. violacea</i>	Ranunculaceae	—	—	—	GenBank	JF422892
<i>Pu. violacea</i>	Ranunculaceae	—	—	—	GenBank	JF422893
<i>Pu. violacea</i>	Ranunculaceae	—	—	—	GenBank	JF422894
<i>Pu. albana</i>	Ranunculaceae	—	—	—	GenBank	JF422890
<i>Pu. dahurica</i>	Ranunculaceae	—	—	—	GenBank	HQ735289
<i>Pu. dahurica</i>	Ranunculaceae	—	—	—	GenBank	GU732648
<i>Pu. halleri</i>	Ranunculaceae	—	—	—	GenBank	FJ639908
<i>Pu. rubra</i>	Ranunculaceae	—	—	—	GenBank	JF422891
<i>Pu. sp.</i>	Ranunculaceae	—	—	—	GenBank	GU732651
<i>Pu. turczaninovii</i>	Ranunculaceae	—	—	—	GenBank	GU732649
<i>Potentilla chinensis</i>	Rosaceae	W1	PS1120W1	leaf	Shengnongjia, Hubei	KR611746
<i>Po. chinensis</i>	Rosaceae	W2	PS1120W2	leaf	Shengnongjia, Hubei	KR611747
<i>Po. chinensis</i>	Rosaceae	W3	PS1120W3	leaf	Shengnongjia, Hubei	KR611748
<i>Po. chinensis</i>	Rosaceae	W4	PS1120W4	leaf	Chongqing	KR611749
<i>Po. chinensis</i>	Rosaceae	W5	PS1120W5	leaf	Chongqing	KR611750
<i>Po. chinensis</i>	Rosaceae	W6	PS1120W6	leaf	Wuhan, Hubei	KR611751
<i>Po. chinensis</i>	Rosaceae	W7	PS1120W7	leaf	Wuhan, Hubei	KR611752
<i>Po. chinensis</i>	Rosaceae	W8	PS1120W8	leaf	Wuhan, Hubei	KR611753
<i>Po. chinensis</i>	Rosaceae	W9	PS1120W9	leaf	Beijing	KR611754
<i>Po. chinensis</i>	Rosaceae	W10	PS1120W10	leaf	Beijing	KR611755
<i>Po. discolor</i>	Rosaceae	F1	PS1079F1	leaf	Beijing	KR611766
<i>Po. discolor</i>	Rosaceae	F2	PS1079F2	leaf	Beijing	KR611767
<i>Po. discolor</i>	Rosaceae	F3	PS1079F3	leaf	Nanning, Guangxi	KR611768
<i>Po. discolor</i>	Rosaceae	F4	PS1079F4	leaf	Nanning, Guangxi	KR611769
<i>Po. discolor</i>	Rosaceae	F5	PS1079F5	leaf	Nanning, Guangxi	KR611770
<i>Po. discolor</i>	Rosaceae	F6	PS1079F6	leaf	Nanning, Guangxi	KR611771
<i>Po. discolor</i>	Rosaceae	F7	PS1079F7	leaf	Nanning, Guangxi	KR611772
<i>Po. discolor</i>	Rosaceae	F8	PS1079F8	leaf	Anhui	KR611773
<i>Rhaponticum uniflorum</i>	Compositae	L1	PS0674L1	leaf	Nanyang, Henan	KR611756
<i>R. uniflorum</i>	Compositae	L2	PS0674L2	root	Beijing	KR611757
<i>R. uniflorum</i>	Compositae	L3	PS0674L3	root	Beijing	KR611758
<i>R. uniflorum</i>	Compositae	L4	PS0674L4	leaf	Beijing	KR611759
<i>R. uniflorum</i>	Compositae	L5	PS0674L5	leaf	Songshan, Henan	KR611760
<i>R. uniflorum</i>	Compositae	L6	PS0674L6	leaf	Songshan, Henan	KR611761
<i>R. uniflorum</i>	Compositae	L7	PS0674L7	leaf	Songshan, Henan	KR611762

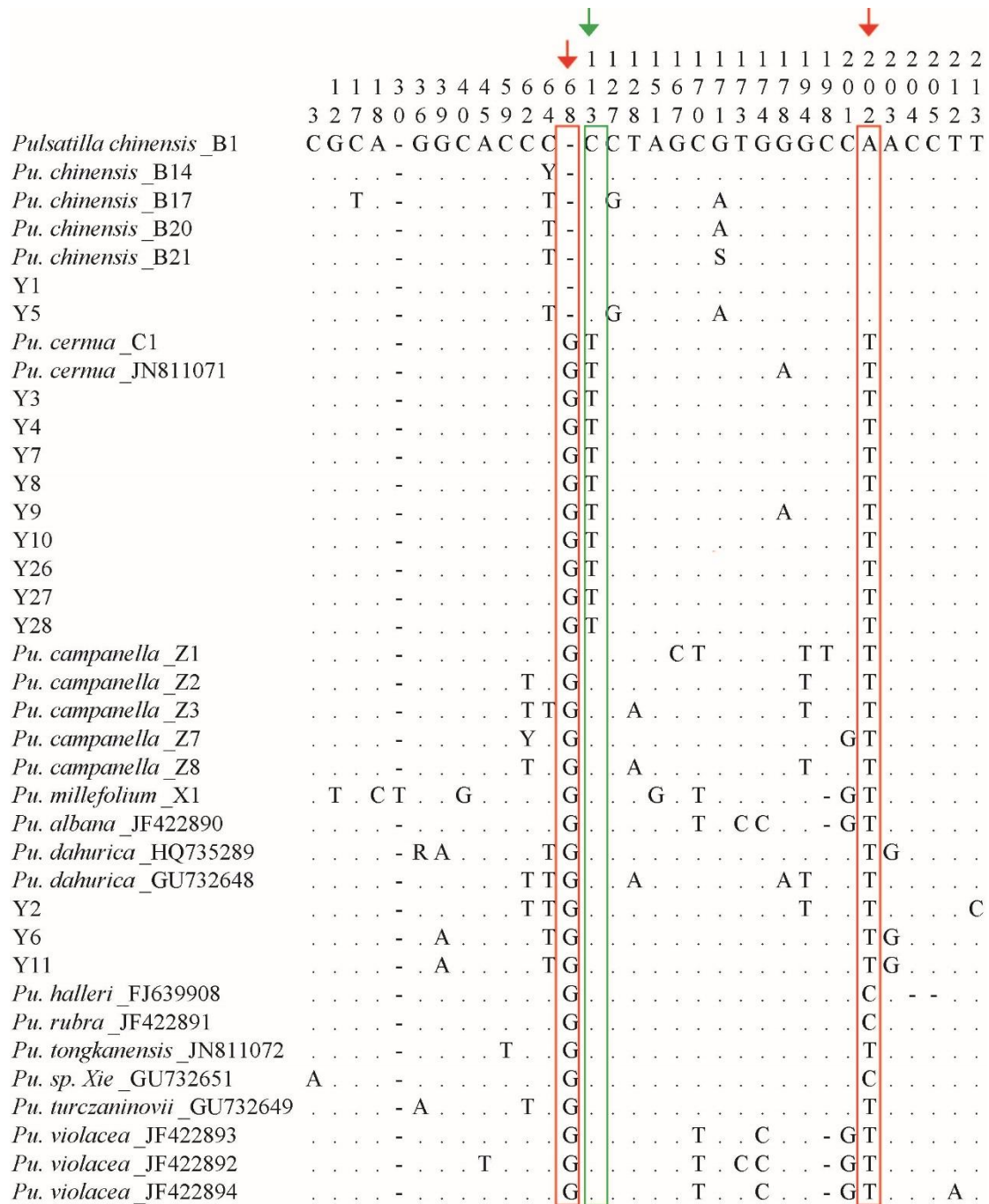
<i>R. uniflorum</i>	Compositae	L8	PS0674L8	leaf	Changbaishan, Jilin	KR611763
<i>R. uniflorum</i>	Compositae	L9	PS0674L9	leaf	Changbaishan, Jilin	KR611764
<i>R. uniflorum</i>	Compositae	L10	PS0674L10	leaf	Changbaishan, Jilin	KR611765
<i>Platycodon grandiflorus</i>	Campanulaceae	J1	PS0583J1	leaf	Qingdao, Shandong	KR611774
<i>P. grandiflorus</i>	Campanulaceae	J2	PS0583J2	leaf	Qingdao, Shandong	KR611775
<i>P. grandiflorus</i>	Campanulaceae	J3	PS0583J3	leaf	Chongqing	KR611776
<i>P. grandiflorus</i>	Campanulaceae	J4	PS0583J4	leaf	Chongqing	KR611777
<i>P. grandiflorus</i>	Campanulaceae	J5	PS0583J5	leaf	Chongqing	KR611778
<i>P. grandiflorus</i>	Campanulaceae	J6	PS0583J6	leaf	Chongqing	KR611779
<i>Ajuga decumbens</i>	Labiatae	JC1	PS1738JC1	leaf	Anhui	KR611783
<i>A. decumbens</i>	Labiatae	JC2	PS1738JC2	leaf	Anhui	KR611784
<i>A. decumbens</i>	Labiatae	JC3	PS1738JC3	leaf	Chongqing	KR611785
<i>A. decumbens</i>	Labiatae	JC4	PS1738JC4	leaf	Yunnan	KR611786
<i>Anemone hupehensis</i>	Ranunculaceae	D1	PS3105D1	leaf	Shengnongjia, Hubei	KR611780
<i>A. hupehensis</i>	Ranunculaceae	D2	PS3105D2	leaf	Shengnongjia, Hubei	KR611781
<i>A. hupehensis</i>	Ranunculaceae	D3	PS3105D3	leaf	Shengnongjia, Hubei	KR611782
<i>A. hupehensis</i>	Ranunculaceae		—	—	GenBank	HQ440207
<i>Leontopodium</i> <i>leontopodioides</i>	Compositae		PS0634MT01	leaf	Donglingshan, Beijing	FJ980329
<i>L. leontopodioides</i>	Compositae		—	—	GenBank	FJ639944
<i>Duhaldea cappa</i>	Compositae		PS0701MT01	leaf	Kunming, Yunnan	FJ980352
<i>D. cappa</i>	Compositae		—	—	GenBank	KP092567
<i>Gerbera piloselloides</i>	Compositae		—	—	GenBank	GU126788
<i>Gnaphalium affine</i>	Compositae		PS0684MT01	leaf	Putian, Fujian	FJ980346

Supplementary Table S2 Intraspecific distances of species in the ITS2 database of *Pulsatilla chinensis* and its adulterants

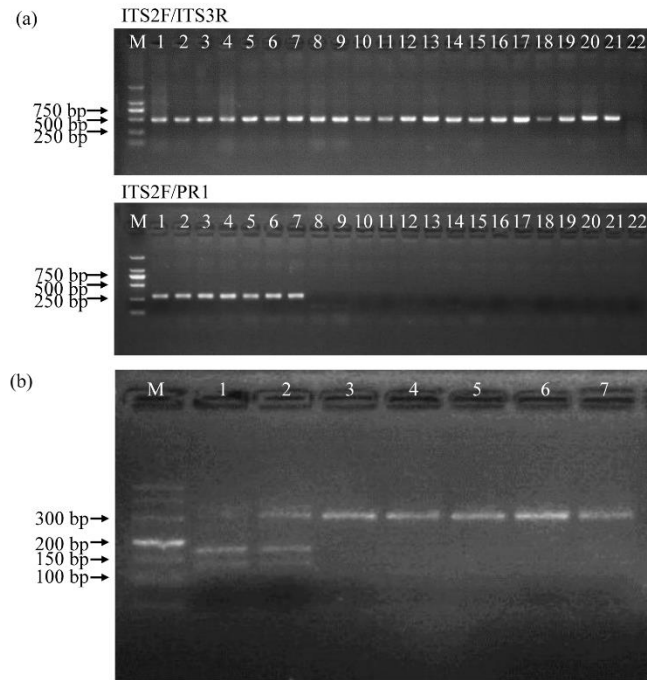
Specie	Sample number	Intraspecific ITS2 distances (KP2)		
		Minimum	Maximum	Mean
<i>Pulsatilla chinensis</i>	25	0	0.019	0.006
<i>Pu. cernua</i>	9	0	0.005	0.002
<i>Pu. campanella</i>	9	0	0.029	0.012
<i>Pu. millefolium</i>	1	—	—	—
<i>Pu. tongkanensis</i>	4	0	0	0
<i>Pu. violacea</i>	3	0.005	0.014	0.010
<i>Pu. dahurica</i>	2	0.029	0.029	0.029
<i>Pu. albana</i>	1	—	—	—
<i>Pu. halleri</i>	1	—	—	—
<i>Pu. rubra</i>	1	—	—	—
<i>Pu. sp.</i>	1	—	—	—
<i>Pu. turczaninovii</i>	1	—	—	—
<i>Potentilla chinensis</i>	10	0	0.078	0.046
<i>Po. discolor</i>	8	0	0.015	0.005
<i>Rhaponticum uniflorum</i>	10	0	0.014	0.007
<i>Platycodon grandiflorus</i>	6	0	0	0
<i>Anemone hupehensis</i>	4	0	0.010	0.004
<i>Ajuga decumbens</i>	4	0	0.005	0.002
<i>Leontopodium leontopodioides</i>	2	0	0	0
<i>Duhaldea cappa</i>	2	0	0	0
<i>Gerbera piloselloides</i>	1	—	—	—
<i>Gnaphalium affine</i>	1	—	—	—

Pulsatilla_chinensis_H1 (15)	1	CACACAGCGTCGCCCCACCAAAGCATTGGATGGGGGCGGAAATTGGCCCCCGAGCCC
Pulsatilla_chinensis_H2 (5)	1	CACACAGCGTCGCCCCACCAAAGCATTGGATGGGGGCGGAAATTGGCCCCCGAGCCC
Pulsatilla_chinensis_H3 (3)	1	CACACAGCGTCGCCCCACCAAAGCATTGGATGGGGGCGGAAATTGGCCCCCGAGCCC
Pulsatilla_chinensis_H4 (1)	1	CACACAGCGTCGCCCCACCAAAGCATTGGATGGGGGCGGAAATTGGCCCCCGAGCCC
Pulsatilla_chinensis_H5 (1)	1	CACACAGCGTCGCCCCACCAAAGCATTGGATGGGGGCGGAAATTGGCCCCCGAGCCC
Pulsatilla_chinensis_H1 (15)	61	CCCGGGCACGGTCGGCACAAATGTTGGCCCTCGGCGGCGAGCGTCGCGGTCAGCGGTGGT
Pulsatilla_chinensis_H2 (5)	61	CCYGGGCACGGTCGGCACAAATGTTGGCCCTCGGCGGCGAGCGTCGCGGTCAGCGGTGGT
Pulsatilla_chinensis_H3 (3)	61	CCTGGGCACGGTCGGCACAAATGTTGGCCCTCGGCGGCGAGCGTCGCGGTCAGCGGTGGT
Pulsatilla_chinensis_H4 (1)	61	CCTGGGCACGGTCGGCACAAATGTTGGCCCTCGGCGGCGAGCGTCGCGGTCAGCGGTGGT
Pulsatilla_chinensis_H5 (1)	61	CCTGGGCACGGTCGGCACAAATGTTGGCCCTCGGCGGCGAGCGTCGCGGTCAGCGGTGGT
Pulsatilla_chinensis_H1 (15)	121	TGTACTCTCATCCTCCAAAGACAAAATGACGCGTCCGCCTCGTCGCCCGCTGGGCGAAGA
Pulsatilla_chinensis_H2 (5)	121	TGTACTCTCATCCTCCAAAGACAAAATGACGCGTCCGCCTCGTCGCCCGCTGGGCGAAGA
Pulsatilla_chinensis_H3 (3)	121	TGTACTCTCATCCTCCAAAGACAAAATGACGCGTCCGCCTCGTCGCCCGCTGGGCGAAGA
Pulsatilla_chinensis_H4 (1)	121	TGTACTCTCATCCTCCAAAGACAAAATGACGCGTCCGCCTCGTCGCCCGCTGGGCGAAGA
Pulsatilla_chinensis_H5 (1)	121	TGTACTCTCATCCTCCAAAGACAAAATGACGCGTCCGCCTCGTCGCCCGCTGGGCGAAGA
Pulsatilla_chinensis_H1 (15)	181	TGACCCAAGGAGTCTCCCAACCGGAGACTT
Pulsatilla_chinensis_H2 (5)	181	TGACCCAAGGAGTCTCCCAACCGGAGACTT
Pulsatilla_chinensis_H3 (3)	181	TGACCCAAGGAGTCTCCCAACCGGAGACTT
Pulsatilla_chinensis_H4 (1)	181	TGACCCAAGGAGTCTCCCAACCGGAGACTT
Pulsatilla_chinensis_H5 (1)	181	TGACCCAAGGAGTCTCCCAACCGGAGACTT

Supplementary Figure S1 Intragenomic variation in the ITS2 sequences of *Pulsatilla chinensis*. The figure was performed by ClustalW-BOXSHADE alignment method. Each digit in the bracket represents the number of the ITS2 haplotype; black shade indicates identical base; gray or white shade indicates variable base; Y represents T/C; S represents C/G.



Supplementary Figure S2 Sequence divergence among *Pulsatilla* species and fourteen commercial Baitouweng products in the ITS2 region. The number represents the positions of variable sites (bp); dots (.) represents the nucleotide identical to the first line; dash (-) represents a nucleotide indel; Y represents T/C; S represents C/G; R represents A/G. Red arrows and boxes indicate the specific SNP sites in *Pu. chinensis*; green arrow and box indicate the specific SNP sites in *Pu. crenua*.



Supplementary Figure S3 Full-length gels of Specific PCR-RFLP assay of *Pulsatilla chinensis* and its adulterants. (a) PCR products by primers ITS2F/ITS3R and ITS2F/PR1. Lane 1-22: *Pu. chinensis* (1-2), *Pu. cernua* (3-4), *Pu. campanella* (5-6), *Pu. millefolium* (7), *Anemone hupehensis* (8-9), *Cimicifuga simplex* (10-11), *Clematis armandii* (12-13), *Potentilla chinensis* (14-15), *Po. discolor* (16-17), *Rhaponticum uniflorum* (18-19), *Platycodon grandiflorus* (20-21) and water control (22). Lane M, DL2000 DNA marker. (b) *Bgl* I restriction digest pattern of PCR products of *Pulsatilla* species. Lane 1-7: *Pu. chinensis* (1-2), *Pu. cernua* (3-4), *Pu. campanella* (5-6) and *Pu. millefolium* (7). Lane M, DL500 DNA marker.