Genetic authentication of Cynanchi Wilfordii Radix and Cynanchi Auriculati Radix by Using Conventional-PCR and Real-time PCR

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Seoul Metropolitan Government Research Institute of Public Health and Environment

2017 Annual Meeting of Korean Society of Food Science and Nutrition

Date: November 8th (Wed) ~ November 10th (Fri), 2017, HICO, GYEONGJU, KOREA

Abstract

The original plant species of Cynanchi Wilfordii Radix belong to the Asclepiadaceae family is differentially described in the national pharmacopoeia of Korea, China and Japan. Dried roots of this plant have been used for prevention and treatment of various diseases in Korea. Owing to the morphological similarities of the dried roots of this plant to those of *Cynanchum auriculatum*, which is often misidentified in Korean herbal medicine marketplace and distinguishing these two species is exceedingly difficult. The aim of this study was to compare the conventional-PCR method with the real-time PCR method for Detection of *Cynanchum wilfordii* and *C. auriculatum* DNA. According to the experimental results, both the conventional-PCR and the real-time PCR method were able to detect the *C. auriculatum* with more than 2% in mixture of *C.wilfordii* and *C. auriculatum.* In the monitoring results of 20 samples, *C.wilfordii* was detected in 19 samples and 1 sample(SP15) was pure *C. auriculatum.* 4 samples was mixture of *C. auriculatum* with *C.wilfordii.* Considering the unintentional mixture(3% or less), there was little difference between the two methods in the case of raw materials. Also in the case of the raw material, the conventional-PCR method detected the *C. auriculatum* at a detection level similar to the real-time PCR, so we suggest that it can be used conventional-PCR based method.

Materials and methods

Materials *Table 1*

- ✓ Samples were collected at random from Seoul Herbal Medicine Market, which were distributed as agricultural products (20 samples)
- ✓ Standard samples (*C.wilfordii* & *C.auriculatum*) received from MFDS

Table 1. Information of Herbal Material for Becksuo Used in This Study

Number of Samples	Locality of Collected Samples	Material
13	Yeongju, Gyeongsangbuk-do	
3	Yeongcheon, Gyeongsangbuk-do	
2	Gangwon-do	Dried Root
1	Sangju, Gyeongsangbuk-do	
1	Not confirmed	

Method

MFDS guideline(1)

Analytical procedure *Figure 1*

- ✓ Sample roots were ground to powder by mixer and made into finer particles using No. 50 sieve (300 μm)
- ✓ The genomic DNA of each sample was extracted according to the manual for DNeasy® Plant Mini Kit (QIAGEN, Germany) and DNA concentration was determined by spectrophotometry (ScanDrop)
- ✓ Extracted DNA was amplified using 7500 Fast Real-Time PCR and Thermal Cycler (AERIS-BD048)

Figure 1. Procedure of PCR analysis

Table 2. Information of species-primer sets used in this study

	Species	Primer Sequences(5'→3')
Conventional PCR	*C. wilfordii*	ATA TTA TAT TCT AAA ATT AGA T CTC TAT TTC TAT TTC TAT
	C. auriculatum	AAA TGA ATT TAA AAA TTC AAT ACA GTT CTA TTT CTA TTT ATT TTT AT
Real-time PCR	PAC	TCT GCC CTA TCA ACT TTC AAT GGT A AAT TTG CGC GCC TGC TGC CTT CCT T
	C. wilfordii	GCG TAT ATG TAG AAA CC CAA AAA AGC CCG TAG
	C. auriculatum	CTT GTT CCA ATT ATT CC AAT GAG AAA AGT TTC TG

Results & Discussion

Sensitivity *Figure 2*

Sensitivity is expressed by Limits of Detection (LOD), which was assessed by analyzing depending on the mixed rate of *C. auriculatum.*

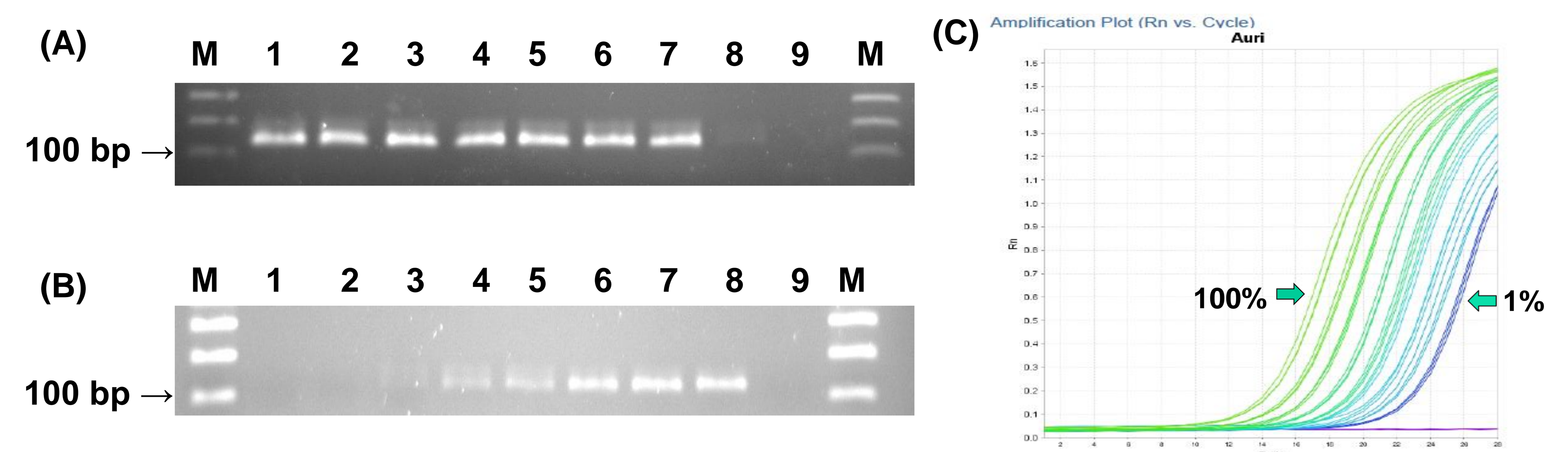

Figure 2. The Conventional-PCR and Real-time PCR detection of *C. auriculatum* adulteration into *C. wilfordii* presented in ratio of 0%, 1%, 2%, 5%, 10%, 20%, 50% and 100% (A) *C. wilfordii* specific primer, (B) *C. auriculatum* specific primer (*C. auriculatum* adulteration ratio 0% (Lane 1), 1% (Lane 2), 2% (Lane 3), 5% (Lane 4), 10% (Lane 5), 20% (Lane 6), 50% (Lane 7), 100% (Lane 8), Negative Control (Lane 9), Size Marker(M), (C) Amplification plot according 1% ~ 100% mixed rate

Monitoring Results of 20 samples in Comparative Analysis *Table 3*

Table 3. Amplification results of samples using Conventional PCR and Real-time PCR

	Conventional-PCR		Real-time PCR		
	C. wilfordii	*C. auriculatum*	PAC	*C. wilfordii*	*C. auriculatum*
SP1	+	-	+	+	-
SP2	+	-	+	+	-
SP3	+	-	+	+	-
SP4	+	-	+	+	-
SP5	+	-	+	+	-
SP6	+	+	+	+	+
SP7	+	-	+	+	-
SP8	+	-	+	+	-
SP9	+	+	+	+	+
SP10	+	-	+	+	-
SP11	+	+	+	+	-
SP12	+	-	+	+	-
SP13	+	-	+	+	-
SP14	+	-	+	+	-
SP15	-	+	+	-	+
SP16	+	-	+	+	-
SP17	+	-	+	+	-
SP18	+	+	+	+	+
SP19	+	-	+	+	-
SP20	+	-	+	+	-

+: Detected, -: Not detected

Conclusion

- ✓ Both the conventional-PCR and the real-time PCR method were able to detect the *C. auriculatum* with more than 2% in mixed samples.
- ✓ *C. wilfordii* was detected in 19 samples and 1 sample(SP15) was pure *C. auriculatum,* 4 samples was mixture of *C. auriculatum* with *C.wilfordii.*
- ✓ In the case of the raw material, the conventional-PCR method detected the *C. auriculatum* at a detection level similar to the real-time PCR

Reference

(1) MFDS Guideline : Detection of *Cynanchum wilfordii and C. auriculatum* DNA using conventional-PCR and real-time PCR(2015, 2016, 2017)

(2) Kyu-Heon Kim et al., : Development of Primer Sets for the Detection of *Polygonum multiflorum*, *Cynanchum wilfordii* and *C. auriculatum. J. Food Hyg. Saf.*, 289-294 (2015)