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ORIGINAL ARTICLE

Development of a Species-Specific Polymerase Chain Reaction-Based Technology for Authentication of Asini Corii Colla and Taurus Corii Colla

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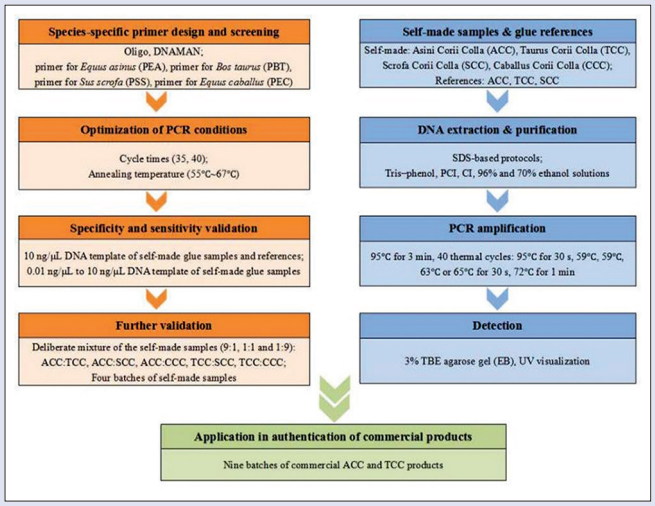
ABSTRACT

Background: Asini Corii Colla (ACC; donkey-hide glue) and Taurus Corii Colla (TCC; bovine-hide glue) are popular healthcare food supplements as well as well-recognized medicine in Traditional Chinese medicine clinics. Due to large demand of them and limited resources of their animal origins, the skin of other animals are often used to make their fake or adulterated products. **Objective:** In this study, we aimed to develop a species-specific polymerase chain reaction (PCR) approach to detect deoxyribonucleic acid (DNA) fragments of ACC and TCC for rapid authentication purpose. **Materials and Methods:** Four sets of novel species-specific primer were designed, and PCR conditions were optimized for the PCR assay, which was further validated for specificity and sensitivity. Then, deliberate mixture was analyzed to further verify the capability of adulteration detection by the developed PCR method. Finally, it was used to assess the authenticity of commercial products. **Results:** Four primers' sets were specific for amplification of extracted mitochondrial DNA of donkey, bovine, swine, and horse when the annealing temperature was 59°C, 59°C, 63°C, and 65°C, and 40 thermal cycles was performed. Under this optimized PCR conditions, 1 ng/μL of horse DNA template has been detected in the sensitivity test, with 0.1 ng/μL for donkey, bovine, and swine. The assay was capable of detecting spiked species at 10% level in premixed ACC or TCC samples. **Conclusion:** The newly developed PCR-based technology was specific and sensitive, and it was a convenient approach for the authentication of commercial ACC and TCC products.

Key words: Adulteration, Asini Corii Colla, authentication, species-specific polymerase chain reaction, Taurus Corii Colla

SUMMARY

- A species-specific polymerase chain reaction (PCR)-based technology was developed to detect intrinsic short deoxyribonucleic acid (DNA) fragments of Asini Corii Colla (ACC) and Taurus Corii Colla (TCC) for rapid authentication purpose. Four designed primer sets were of high specificity for their corresponding species, which can be used for identification of animal origins in glue products regardless of their similar morphological characteristics. The sensitivity for primers was determined as 1 ng/μL or lower. This PCR method was capable to detect 10% adulteration of bovine, swine, or horse ingredients in ACC products and swine or horse ingredients in TCC products. The new technology needs nonsequencing rather than DNA barcoding, and it could be further improved for authentication of Chinese patent medicine made from ACC and TCC.



Abbreviations used: DNA: Deoxyribonucleic acid; PCR: Polymerase chain reaction; bp: Base pair.

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INTRODUCTION

Traditional Chinese medicine (TCM) has a history of 2000 years, and it has been strongly recommended by the World Health Organization as an important step toward its long-term goal of universal health care since more and more modern pharmacology research demonstrated its significant curative effects in clinics.[1-4]

Gelatinous Chinese medicines (GCMs) are a very important kind of animal-derived Chinese medicine (ACM) and extensively used as hemopoietic and hemostatic medicines to treat dizziness, palpitation, and insomnia in TCM clinics. They are also consumed

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as a popular tonic for weak people, the elderly, or puerpera.[5-7] In recent decades, due to large demand of GCMs as well as the limited resources of their animal origins, bovine skin was often used to make fake or adulterated Asini Corii Colla (ACC; donkey-hide glue) and even cheaper swine skin was added during the production of Taurus Corii Colla (TCC; cattle-hide glue) too.[8,9] The other two fake products including Scrofa Corii Colla (SCC; swine-hide glue) and Caballus Corii Colla (CCC; horse-hide glue) are often misused. This situation exposes the public health to a high risk and causes unfair competitions in the commercial market. Therefore, the establishment of a reliable and convenient technology is of great importance for the identification of their animal origins in ACC and TCC products.[10-12] So far, various spectroscopy-based and chromatographic methods such as two-dimensional correlation infrared spectroscopy, near-infrared spectroscopy, reversed-phase high-performance liquid chromatography, and gas chromatography–mass spectrometry have been studied for quality control of ACMs.[13-18] However, similar chemical properties always make it difficult to accurately identify species in a adulterant, and they have not reached the stage of practical application due to sample-processing variability.[19]

Deoxyribonucleic acid (DNA)-based molecular biomarkers have been widely applied to authenticate species for health, economic, and religious purposes.[20-22] Moreover, polymerase chain reaction (PCR)-based assays were often employed in species identification and gained more and more research interests in recent years.[23-28] Mitochondrial DNA (*mt*DNA) has been found to be better suited for species identification and authentication than other genetic markers like cell nucleus DNA since *mt*DNA is several fold more abundant than that of nuclear genome; meanwhile, multicopy *mt*DNA has a higher chance of survival in processed products than single-copy nuclear DNA, contributing to improve the assay sensitivity.[29-35] In this piece of work, four sets of novel primer were designed for species-specific PCR assay on the basis of the differences in DNA sequences of donkey, bovine, swine, and horse. Then, thermal cycles and annealing temperature were optimized for the PCR assay, which was validated for specificity and sensitivity. Eventually, an effective and convenient PCR-based technology was developed for authentication of commercial ACC and TCC products.

MATERIALS AND METHODS

Samples

Four types of glue sample were self-made from the skin of donkey (*Equus asinus*), bovine (*Bos taurus*), swine (*Sus scrofa*), and horse (*Equus caballus*) in our laboratory. Four batches of glue references including two batches of ACC, one batch of TCC, and another one batch of SCC were purchased from the National Institutes for Food and Drug Control (PRC). In addition, nine batches of commercial ACC and TCC products made by different manufactures were collected from drug stores, hospitals, and markets. Their information was detailed in Table 1.

Deoxyribonucleic acid extraction

The extraction of DNA fragments from the above samples was performed using Sodium dodecyl sulfate-based protocols. 50 mg of each sample was mixed with 990 μL of extraction buffer and 10 μL of proteinase K in a 1.5 mL centrifuge tube. After incubation at 56°C for 6 h, the tube was centrifuged at 12000 rpm for 15 min. The supernatant was transferred into a new clear tube, and an equal volume of Tris-phenol solution, PCI solution, and CI solution was sequentially mixed with the supernatant for further DNA purification. Then, the resulting supernatant was precipitated by two volumes of 96% ethanol and 0.1 volume of 5.0 M KAc solution. After centrifugation at 12000 rpm for 15 min, the DNA pellet was collected and washed with 70% ethanol and finally reconstituted in 20 μL of TE buffer (pH 8.0) for subsequent PCR experiments. These DNA extracts were quantified by ultraviolet (UV) spectrophotometry at 260 nm and 280 nm on a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and then stored at −20°C until use.

Table 1: Glue references and commercial products used in this study

Code	Products	Sources	Batch number
R1	ACC	NIFDC	121274-201202
R2	ACC	NIFDC	121274-201703
R3	TCC	NIFDC	121695-201301
R4	SCC	NIFDC	121274-201701
D1	ACC	Shandong Dong'E Co., Ltd	1605024
D2	ACC	Shandong Dong'E Co., Ltd	1508041
D3	ACC	Shandong Dong'E Co., Ltd	1711037
D4	ACC	Shandong Dong'E Co., Ltd	1407049
D5	ACC	Shandong JiShui Co., Ltd	20150916
D6	ACC	Shandong JiShui Co., Ltd	20160918
C1	TCC	Shandong Dong'E Co., Ltd	1408016
C2	TCC	-	B140908
C3	TCC	SaiXing Co., Ltd	180204

NIFDC: National Institutes for Food and Drug Control; ACC: Asini Corii Colla; TCC: Taurus Corii Colla

Primers' design

Four sets of species-specific primers were designed using Oligo (v. 7.60) according to mitochondrial genome sequences of four species. Considering the deep processing degree of the animal origins, the primers for target fragment shorter than 100 bp were selected. The primer sets were evaluated by DNAMAN (v. 8.0.8.789); then, all of them were synthesized by Sangon Biotech (Shanghai) Co., Ltd., and kept at −20°C before use. Information of species-specific primers was summarized in Table 2.

Polymerase chain reaction conditions

PCR amplification was carried out in a 25 μL total reaction mixture composed of 2.5 μL of ×10 PCR buffer, 2.0 mM of $MgCl_2$, 0.2 mM of dNTP, 0.2 μM of forward and reverse primer, 0.625 unit of Taq polymerase, 19.875 μL of ultrapure water, and 1 μL of DNA template (diluted to a final concentration of 10 ng/μL). After an initial denaturation step at 95°C for 3 min, 35 or 40 thermal cycles were performed on a Biorad T100 Thermal Cycler according to the following program: melting at 95°C for 30 s, annealing at 55°C ~61°C, 55°C ~61°C, 59°C ~65°C, and 61°C ~67°C for the detection of donkey, bovine, swine, and horse DNA, respectively, 30 s extension at 72°C for 1 min with a final extension step at 72°C for 7 min. After amplification, 10 μL of the resulting PCR amplicons was analyzed by electrophoresis on a 3% TBE agarose gel containing ethidium bromide in ×0.5 TBE buffer and visualized by UV transillumination.

Validation for specificity and sensitivity

Since DNA extracted from glue samples has been highly degraded to short fragments during the production, two thermal cycles were optimized to obtain a positive result after amplification of the DNA

templates. Annealing temperature was also optimized for good specificity of the primer sets. During the establishment of an effective species-specific PCR method for the detection of animal origins of ACC and TCC products, PCR conditions were optimized on 10 ng/μL DNA of self-made glue samples and the method was then validated for specificity and sensitivity.

RESULTS AND DISCUSSION

Optimized polymerase chain reaction conditions

Extra thermal cycles were needed to achieve bright bands of the positive result after amplification of the short DNA fragments; therefore, 40 thermal cycles were more suitable for the PCR assay. Then, the proper annealing temperature was determined as 59°C, 59°C, 63°C, and 65°C for the detection of donkey, bovine, horse, and swine DNA, respectively.

Specificity of the species-specific primers

Investigation of the specificity of these primer sets was performed on 10 ng/μL DNA template of self-made glue samples and references. As shown in Figures 1 and 2, the expected PCR amplicons were only produced from the target species; four bands at 71 bp, 76 bp, 68 bp, and 75 bp were visualized as predicted and no cross-reactivity with the DNA from other species was observed. Furthermore, amplicons were not found from negative controls after PCR assay by any of these four species-specific primer sets. These validation results suggested that the designed primers were of high specificity for their

Table 2: Species-specific primer sets used for polymerase chain reaction assay in this study

Species	Code	Sequence (5′-3′)	Amplicon size (bp)	Accession number
Equus asinus	PEA	F TATTTCCTATTTGCTTACGCCAT R AGGATAAGGGCTAATACACCAC	71	NC_001788.1
Bos taurus	PBT	F AACACCCATATATCACCATCGGAC R GCCGTTGGTATTAGCACTAGGAT	76	NC_006853.1
Sus scrofa	PSS	F CAACAGCTTTCTCATCAGTTACAC R TAGATAGCGAATAACTCATCCGT	68	NC_012095.1
Equus caballus	PEC	F CACATAACACCATACCCACCT R GGCAGACAATTTAATGCACGAC	75	NC_001640.1

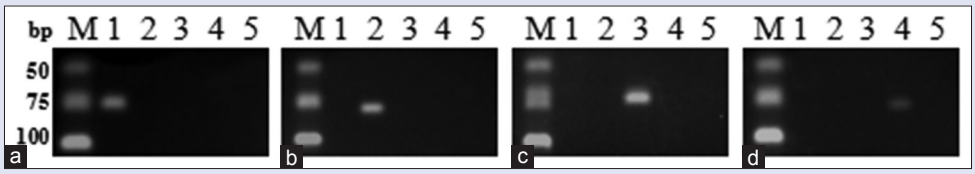

Figure 1: Specificity test on self-made samples for four designed primer sets. (a) PEA; (b) PBT; (c) PSS; (d) PEC; M: Deoxyribonucleic acid marker; Lane 1: Asini Corii Colla; Lane 2: Taurus Corii Colla; Lane 3: SCC; Lane 4: CCC; Lane 5: Negative control

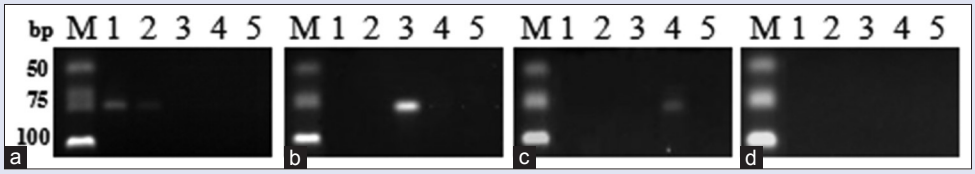

Figure 2: Specificity test on references for four designed primer sets. (a) PEA; (b) PBT; (c) PSS; (d) PEC; M: Deoxyribonucleic acid marker; Lane 1~2: Asini Corii Colla; Lane 3: Taurus Corii Colla; Lane 4: SCC; Lane 5: Negative control

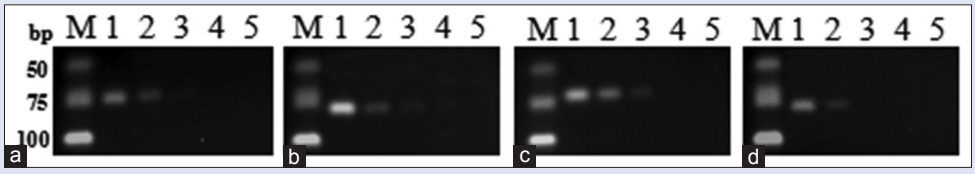

Figure 3: Sensitivity test for the specific primer sets. (a) PEA; (b) PBT; (c) PSS; (d) PEC; M: Deoxyribonucleic acid marker; Lane 1~4: 10 ng/μL, 1 ng/μL, 0.1 ng/μL, 0.01 ng/μL of Deoxyribonucleic acid template; Lane 5: Negative control

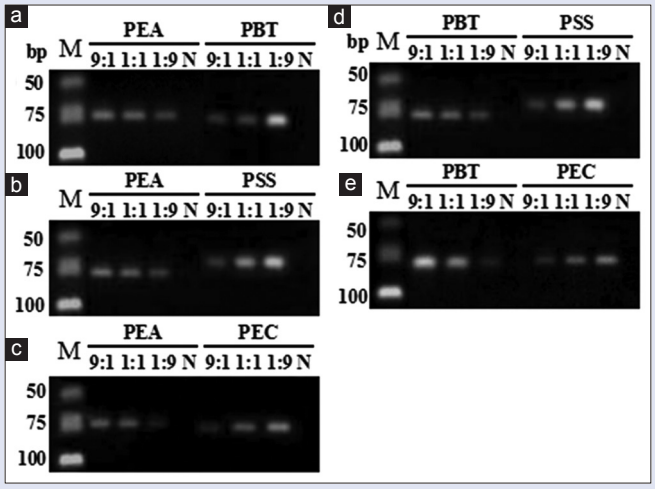

Figure 4: Species-specific polymerase chain reaction assay for self-made sample mixtures. (a) Asini Corii Colla mixed with Taurus Corii Colla; (b) Asini Corii Colla mixed with SCC; (c) Asini Corii Colla mixed with CCC; (d) Taurus Corii Colla mixed with SCC; (e) Taurus Corii Colla mixed with CCC; M: Deoxyribonucleic acid marker; N: Negative control

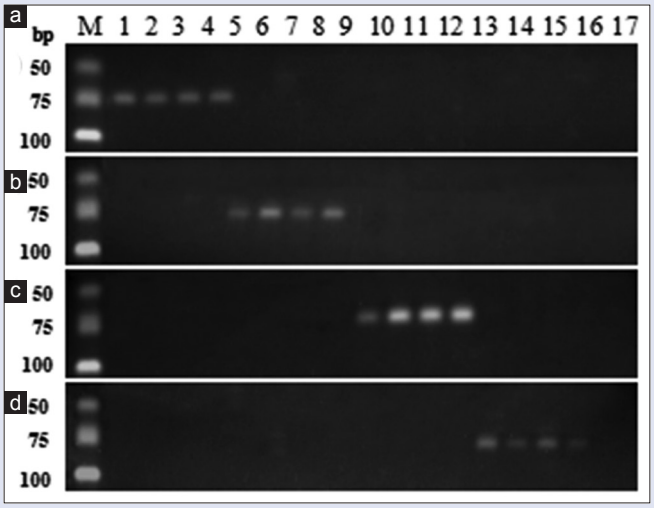

Figure 5: Analysis of four batches of self-made samples by the specific primer sets. (a) PEA; (b) PBT; (c) PSS; (d) PEC; M: Deoxyribonucleic acid marker; Lane 1~4: Asini Corii Colla; Lane 5~8: Taurus Corii Colla; Lane 9~12: SCC; Lane 13~16: CCC; Lane 17: Negative control

corresponding species, which can be used for identification of animal origins in glue products regardless of their similar morphological characteristics.

Sensitivity of the polymerase chain reaction assay

The sensitivity of each primer set against their corresponding species was investigated on the DNA template extracted from self-made sample, and a series of concentration ranging from 0.01 ng/μL to 10 ng/μL was subject to the PCR amplification. As shown in Figure 3, clear bands have been achieved after the amplification against PEA, PBT, PSS, and PEC when the DNA concentration of these species was 1 ng/μL or above. Furthermore, PEA, PBT, and PSS were even able to generate a band against 0.1 ng/μL of donkey, bovine, and swine DNA template. Consequently, the sensitivity for PEC was determined as 1 ng/μL for horse DNA template, while the other three primer sets have achieved higher sensitivity, as low as 0.1 ng/μL.

To further validate the capability of adulteration detection by the developed PCR method, deliberate mixture of the self-made samples was tested. In details, ACC was mixed with TCC, SCC, or CCC; meanwhile, TCC was spiked into SCC or CCC in three levels (9:1, 1:1, and 1:9), with a total weight of 50 mg per mixed sample. As shown in Figure 4, four corresponding species have been all successfully detected after DNA extracted from the mixtures was subject to the species-specific PCR assay. Therefore, this PCR method was capable to detect 10% adulteration of bovine, swine, or horse ingredients in ACC products and swine or horse ingredients in TCC products.

Analysis of self-made samples

To further investigate the reliability of the PCR method, four batches of self-made samples were subject to DNA extraction, and then, they were amplified by the species-specific primer sets. The results were shown in Figure 5; it can be seen that four corresponding species in self-made glue samples were detected, respectively, without any other visible nonspecific bands. Consequently, the newly developed species-specific PCR method was validated to be reliable for the detection of donkey, bovine, swine, and horse ingredients in glue products.

Table 3: Results of species identification in nine commercial products

Code	PEA	PBT	PSS	PEC
D1	+	+	+	-
D2	+	-	-	-
D3	+	+	-	-
D4	+	-	-	-
D5	+	-	-	-
D6	+	-	+	-
C1		+	-	-
C2		-	+	-
C3		-	-	-

The PCR results are reported as positive (+) or negative (-). PCR: Polymerase chain reaction

Application of species-specific polymerase chain reaction assay to commercial products

The established species-specific PCR assay was applied to test nine batches of commercially available ACC and TCC products. The resulting gel electrophoregrams were shown in Figure 6, and it can be seen that eight out of nine batches were successfully amplified to form a clear band under the optimized species-specific PCR conditions except for batch C3. In addition, none of horse ingredients were detected for adulteration in any of the tested products. Among six batches of ACC products, adulteration with bovine ingredients were found in batch D1 and D3, and adulteration with swine ingredients was also found in batch D1 and D6. Among three batches of TCC products, adulteration with swine ingredients was detected in batch C2; however, no visual band has been observed after the PCR assay on batch C3, suggesting that this batch of TCC product could be made from the skin of other animals. The testing results were summarized in Table 3.

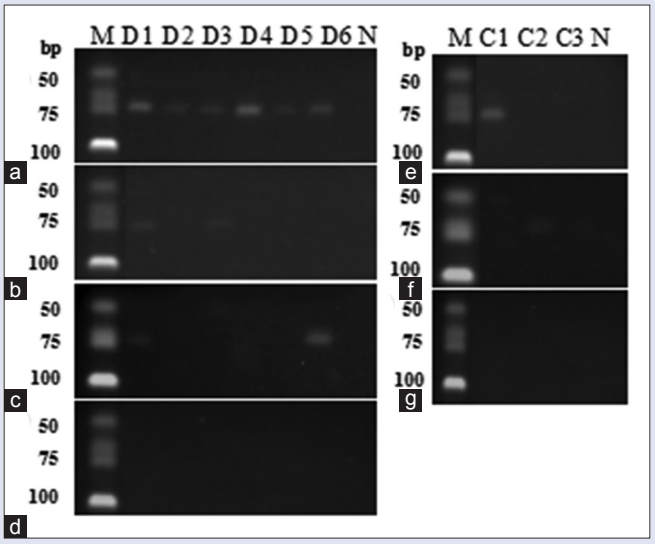

Figure 6: Analysis of nine commercial products by species-specific primer sets. (a) PEA; (b and e) PBT; (c and f) PSS; (d and g): PEC; M: Deoxyribonucleic acid marker; N: Negative control

CONCLUSION

In this study, a species-specific PCR-based technology was newly established for the identification of donkey, bovine, swine, and horse ingredients in commercial ACC and TCC products. It was found that skin of bovine and swine was used to make adulterated ACC products, while swine skin was used to make fake TCC products. Compared to the conventional DNA barcoding, this method was more convenient and time-saving as no sequencing procedure and it was applicable for adulterated glue products. The developed technology was significant from the viewpoint of public health and fair competition in market, and it could be further improved for authentication of Chinese patent medicine made from ACC and TCC.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Hao P, Jiang F, Cheng J, Ma L, Zhang Y, Zhao Y, *et al.* Traditional Chinese medicine for cardiovascular disease: Evidence and potential mechanisms. J Am Coll Cardiol 2017;69:2952-66.
2. Efferth T, Li PC, Konkimalla VS, Kaina B. From traditional Chinese medicine to rational cancer therapy. Trends Mol Med 2007;13:353-61.
3. Mu J, Liu T, Jiang L, Wu X, Cao Y, Li M, *et al.* The traditional Chinese medicine baicalein potently inhibits gastric cancer cells. J Cancer 2016;7:453-61.
4. Cyranoski D. Why Chinese medicine is heading for clinics around the world. Nature 2018;561:448-50.
5. Yang H, Shen Y, Xu Y, Maqueda AS, Zheng J, Wu Q, *et al.* A novel strategy for the discrimination of gelatinous Chinese medicines based on enzymatic digestion followed by nano-flow liquid chromatography in tandem with orbitrap mass spectrum detection. Int J Nanomedicine 2015;10:4947-55.
6. Zong Y, Wang Y, Li H, Li N, Zhang H, Sun J, *et al.* Simultaneous quantification and splenocyte-proliferating activities of nucleosides and bases in Cervi Cornu Pantotrichum. Pharmacogn Mag 2014;10:391-7.
7. Yang H, Zheng J, Wang HY, Li N, Yang YY, Shen YP. Comparative proteomic analysis of three gelatinous Chinese medicines and their authentications by tryptic-digested peptides profiling using matrix-assisted laser desorption/Ionization-time of flight/Time of flight mass spectrometry. Pharmacogn Mag 2017;13:663-7.
8. Wang D, Ru W, Xu Y, Zhang J, He X, Fan G, *et al.* Chemical constituents and bioactivities of Colla Corii Asini. Drug Discov Ther 2014;8:201-7.
9. Sun DY, Zhao WF. Determination of 6 harmful elements in cattle-hide glue by microwave digestion-ICP-MS. Chin J Modern Appl Pharm 2015;32:1227-30.
10. Yang Y, He J, Huang Z, Zhong N, Zhu Z, Jiang R, *et al.* Analysis of hexavalent chromium in Colla Corii Asini with on-line sample pretreatment valve-switching ion chromatography. J Chromatogr A 2013;1305:171-5.
11. Wu H, Ren C, Yang F, Qin Y, Zhang Y, Liu J, *et al.* Extraction and identification of collagen-derived peptides with hematopoietic activity from Colla Corii Asini. J Ethnopharmacol 2016;182:129-36.
12. Kumeta Y, Maruyama T, Asama H, Yamamoto Y, Hakamatsuka T, Goda Y. Species identification of Asini Corii Collas (donkey glue) by PCR amplification of cytochrome b gene. J Nat Med 2014;68:181-5.
13. Xu CH, Zhou Q, Sun SQ, Wang BQ. The identification of Ejiao by two-dimensional correlation infrared spectroscopy. Chin J Anal Chem 2005;33:221-4.
14. Qu HB, Yang HL, Cheng YY. Fast and nondestructive discrimination of donkeyhide glue by near-infrared spectroscopy. Guang Pu Xue Yu Guang Pu Fen Xi 2006;26:60-2.
15. Chen PH, Wang SF, Tian SS, Gong XC, Zhang Y. Determination of 13 amino acids in Asini Corii Colla by RP-HPLC with precolumn derivatization. Chin Tradit Herbal Drugs 2013;44:1995-9.
16. Mao GN, Guo Q, Li X, Qu JB. Anaysis of flavor ingredients in Colla Corii Asini with GC-MS. Prog Vet Med 2010;31:72-5.
17. Weon JB, Yang HJ, Ma JY, Ma CJ. A HPLC-DAD method for the simultaneous determination of five marker components in the traditional herbal medicine bangpungtongsung-san. Pharmacogn Mag 2011;7:60-4.
18. Zhang Y, Xue K, Zhao EY, Li Y, Yao L, Yang X, *et al.* Determination of oleanolic acid and ursolic acid in Chinese medicinal plants using HPLC with PAH polymeric C_{18}. Pharmacogn Mag 2013;9:S19-24.
19. Zuo HL, Zhao J, Wang YT, Xia ZN, Hu YJ, Yang FQ. Identification of the adulterated Asini Corii Colla with cytochrome c oxidase subunit I gene-based polymerase chain reaction. Pharmacognosy Res 2017;9:313-8.
20. Nithaniyal S, Vassou SL, Poovitha S, Raju B, Parani M. Identification of species adulteration in traded medicinal plant raw drugs using DNA barcoding. Genome 2017;60:139-46.
21. Kermani F, Shams-Ghahfarokhi M, Gholami-Shabani M, Razzaghi-Abyaneh M. Diversity, molecular phylogeny and fingerprint profiles of airborne *Aspergillus* species using random amplified polymorphic DNA. World J Microbiol Biotechnol 2016;32:96.
22. Yang H, Yu P, Lu Y, Jiao Z, Chen L, Zhou Y, *et al.* A novel non-sequencing approach for rapid authentication of testudinis carapax et plastrum and trionycis carapax by species-specific primers. R Soc Open Sci 2018;5:172140.
23. Yentur Doni N, Yildiz Zeyrek F, Seyrek A. Detection of plasmodium using filter paper and nested PCR for patients with malaria in Sanliurfa, in Turkey. Malar J 2016;15:299.
24. Josefsen L, Christiansen SK. PCR as a tool for the early detection and diagnosis of common bunt in wheat, caused by *Tilletia tritici*. Mycol Res 2002;106:1287-92.
25. Taghadosi V, Hosseinzadeh S, Shekarforoush SS, Samiei A. Prevalence of renal lesions in slaughtered cattle in Shiraz, Iran, and detection of *Leptospira* in them by nested PCR-RFLP. Trop Anim Health Prod 2016;48:1691-6.
26. Yang X, Hameed U, Zhang AF, Zang HY, Gu CY, Chen Y, *et al.* Development of a nested-PCR assay for the rapid detection of Pilidiella granati in pomegranate fruit. Sci Rep 2017;7:40954.
27. Yang H, Zhou Y, Yu P, Yang Y, Jiao Z, Tam JP, *et al.* A novel PCR-based technology for rapid and

non-sequencing authentication of *Bombyx* batryticatus using species-specific primers. Nat Prod Res 2019;33:1251-6.

28. Yang Y, Zheng Y, Lu B, Jiao Z, Chen L, Gblinwon RT, *et al*. Rapid identification of cervus antlers by species-specific PCR assay. Nat Prod Res 2019. DOI: 10.1080/14786419.2018.1560285.
29. Tobe SS, Linacre AM. A multiplex assay to identify 18 European mammal species from mixtures using the mitochondrial cytochrome b gene. Electrophoresis 2008;29:340-7.
30. Chen TT, Wang MJ, Jiang SY, Xiong SQ, Zhu DC, Wei H. Investigation of the microbial changes during koji-making process of Douchi by culture-dependent techniques and PCR-DGGE. Int J Food Sci Tech 2011;46:1878-83.
31. Kim MJ, Kim HY. Species identification of commercial jerky products in food and feed using direct pentaplex PCR assay. Food Control 2017;78:1-6.
32. Zheng Y, Lu B, Yang Y, Jiao Z, Chen L, Yu P, *et al*. Rapid identification of medicinal leech by species-specific PCR technology. Pharmacogn Mag 2019;15:410-5.
33. Duan N, Gong W, Wu S, Wang Z. Selection and application of ssDNA aptamers against clenbuterol hydrochloride based on ssDNA library immobilized SELEX. J Agric Food Chem 2017;65:1771-7.
34. Li Y, Sun L, Qian J, Long L, Li H, Liu Q, *et al*. Fluorescent "on-off-on" switching sensor based on CdTe quantum dots coupled with multiwalled carbon nanotubes@graphene oxide nanoribbons for simultaneous monitoring of dual foreign DNAs in transgenic soybean. Biosens Bioelectron 2017;92:26-32.
35. Zhu W, Xuan C, Liu G, Chen Z, Wang W. A label-free fluorescent biosensor for determination of bovine serum albumin and calf thymus DNA based on gold nanorods coated with acridine orange-loaded mesoporous silica. Sensor Actuat B Chem 2015;220:302-8.