

市售中药饮片DNA条形码鉴定研究

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摘要: 中药材DNA条形码分子鉴定法指导原则已纳入《中国药典》, 但目前尚缺乏应用于中药饮片的系统性研究。本研究共收集212份市售中药饮片, 包含根及根茎、果实种子、全草、花、叶、皮、茎木等不同入药部位, 验证中药材DNA条形码分子鉴定法对中药饮片基原物种鉴定的适用性和准确性。结果表明, 共有161份样本成功获得DNA条形码序列, 占收集中药饮片样本量的75.9%, 其余样本基因组DNA降解, 无法获得扩增产物。实验所得DNA条形码序列分析结果如下: 138份饮片为《中国药典》(2020年版) 收载相应药材的基原物种, 占获得序列样本数的85.7%; 14份样本分析结果中包含《中国药典》收载相应药材的基原物种以及同属近缘物种, 经过形态学鉴定, 其中8份为正品, 3份掺有伪品, 3份缺少鉴别特征, 不能确定基原; 7份样本为混伪品; 另有2份样本检出掺伪现象。本研究证实中药材DNA条形码分子鉴定法能有效检出市售中药饮片的掺伪/混伪现象, 可用于市售中药饮片的基原物种鉴定, 值得在中药饮片加工生产企业及药品监督检验管理机构推广, 同时有助于促进中药学研究的跨学科交流, 共同挖掘中药作用靶点和药效机制, 推动个性化精准医疗及“精准药物研发”。

关键词: 中药饮片; DNA条形码; ITS2; *psbA-trnH*; 分子鉴定; 市场监控

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DNA barcoding identification of commercial decoctions in traditional Chinese medicine

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Abstract: Although the guiding principles for molecular identification of traditional Chinese medicines (TCM) using DNA barcoding have been recorded in the Chinese Pharmacopoeia, there is still a lack of systematic research on its application to commercial TCM decoctions. In this study, a total of 212 commercial TCM decoctions derived from different medicinal parts such as root and rhizome, fruit and seed, herb, flower, leaf, cortex, and

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caulis were collected to verify applicability and accuracy of the method. DNA barcodes were successfully obtained from 75.9% (161/212) of the samples, while other samples failed to be amplified due to genomic DNA degradation. Among the 161 samples, 85.7% of them were identified as recorded species in the Chinese Pharmacopoeia (2020 edition). In addition, 14 samples could be identified as species recorded in the Chinese Pharmacopoeia and their closely related species in the same genus. Morphological identification for the unconfirmed samples showed that eight were genuine species and three were adulterants, while the other three were unidentifiable due to lack of morphological characteristics. Furthermore, the DNA barcodes of seven samples accurately mapped to the sequences of adulterants. Remarkably, counterfeit products were detected in two samples. These results demonstrate that DNA barcoding is suitable for the identification of commercial TCM decoctions. The method can effectively detect adulterants and is appropriate for use throughout the industrial chain of TCM production and distribution, and by the supervisory agencies as well.

Key words: decoction pieces; DNA barcoding; ITS2; *psbA-trnH*; molecular identification; market supervision

中药饮片质量关乎临床用药安全,近年来各级药品监管部门持续加大对中药饮片监督检查和抽检力度,依法查处和曝光违法违规企业和不合格产品,中药饮片总体质量状况有所好转,2019年全国中药材及饮片质量分析报告显示,当年全国平均抽检合格率为91%,较之前有所提升,但仍存在掺伪掺杂现象,如金银花与山银花、金钱草与广金钱草、木通与川木通、五加皮与香加皮等,此外,还存在染色剂增重、过度硫熏、虫蛀霉变等现象,严重影响中药材及饮片质量^[1]。国家药品监督管理局定期发布“关于药品不符合规定的通告”,2020年1~10月共发文5次,总计通报98批次药品不符合规定,其中中药材及饮片27批次,占比27.55%,主要不符合规定的项目为性状、鉴别、含量测定等,潜在影响临床用药安全。

“中药材DNA条形码分子鉴定法指导原则”自2014年首次被收载于《中国药典》(2010年版第三增补本)^[2]至今已有6年,在各省市区药品检验机构得到一定程度的推广应用。《中国药典中药材DNA条形码标准序列》一书为《中国药典》(2015年版一部)收载的动、植物药材提供了标准DNA条形码序列^[3],是采用DNA条形码技术鉴定中药材基原物种的重要依据。现行版《中国药典》(2020年版四部)除“中药材DNA条形码分子鉴定法指导原则”^[4]外,还收载“DNA测序技术指导原则”^[5]、“标准核酸序列建立指导原则”^[6]等,显示分子鉴定方法在《中国药典》中所占比重逐渐加大。中药材DNA条形码分子鉴定法在中药材基原物种鉴定方面已有大量实践应用报道,如羌活^[7]、秦艽^[8]、蛤蟆油^[9]等,亦有报道采用DNA条形码技术鉴定鹿茸饮片^[10]及中药破壁饮片^[11,12],但对于原料药材加工制成的饮片是否适用,尚缺乏系统文献报道,因此本研究拟对市售中药饮片基原物种进行鉴定,验证该方法对中药饮片基原物种鉴定的适用性和准确性。

材料与方法

材料 自药店购买常见中药饮片,每种饮片1~2份,按不同入药部位归类,其中根及根茎73份,果实种子52份,全草45份,花15份,叶11份,皮6份,茎木4份,其他(包括树脂、藻菌地衣等)6份,共计212份,编号为JCS001~JCS212,样品信息详见表1。

DNA提取 参考《中国药典中药材DNA条形码标准序列》一书^[3],对各饮片样本进行前处理,如:①根及根茎类、茎木类饮片需用75%乙醇擦拭药材表面后晾干,刮去外部栓皮,取内部的饮片切成约2 mm³的小块;②部分饮片多糖、多酚含量较高,需加入样品量10%的PVP-40后再进行研磨;③部分饮片多糖、色素、脂肪含量较高,在研磨后需采用核分离液漂洗。依照《中国药典》(2020年版四部)收载“中药材DNA条形码分子鉴定法指导原则”^[4]规定的不同入药部位DNA提取标准操作流程进行实验,提取各饮片样本基因组DNA。

DNA条形码序列获得 DNA条形码序列扩增、测序及数据处理均依照“中药材DNA条形码分子鉴定法”标准操作流程(DNA barcoding SOP)进行。参考《中国药典中药材DNA条形码标准序列》一书,部分物种ITS2序列难以获取,故扩增其*psbA-trnH*序列^[3]。ITS2序列、*psbA-trnH*序列通用引物及反应程序见表2。

物种判定 参照《中国药典》(2020年版四部)收载标准操作流程及前期文献^[4,13]报道,采用“中药材DNA条形码鉴定系统(<http://www.tcmbarcodes.cn>)”进行结果判定。对于不能获得DNA条形码序列或DNA条形码分子鉴定结果存在多个同属近缘物种的样本,由中国医学科学院药用植物研究所标本馆林余霖研究员进行性状鉴定,判定其基原物种。

Table 1 Sample list and DNA barcoding identification results. "/" referred to genomic DNA degradation and no PCR product; "MI" referred to morphological identification

Sample No.	Lable name	Medicinal part	Nearest match	ITS2	<i>psbA-trnH</i>
JCS059	Baizhu	Root and rhizome	<i>Atractylodes macrocephala</i>	√	
JCS060	Baitouweng	Root and rhizome	<i>Pulsatilla chinensis</i>	√	
JCS061	Baixianpi	Root and rhizome	<i>Dictamnus dasycarpus</i>	√	
JCS066	Beidougen	Root and rhizome	<i>Menispermum dauricum</i>	√	
JCS024	Beishashen	Root and rhizome	<i>Glehnia littoralis</i>	√	
JCS037	Cangzhu	Root and rhizome	<i>Atractylodes lancea</i>	√	
JCS072	Chaihu	Root and rhizome	<i>Bupleurum chinense</i>	√	
JCS073	Changpu	Root and rhizome	<i>Acorus tatarinowii</i>	√	
JCS075	Chishao	Root and rhizome	<i>Paeonia lactiflora</i>	√	
JCS010	Danshen	Root and rhizome	<i>Salvia miltiorrhiza</i>	√	
JCS043	Danggui	Root and rhizome	<i>Angelica sinensis</i>	√	
JCS088	Duhuo	Root and rhizome	<i>Angelica pubescens f. biserrata</i>	√	
JCS092	Fangfeng	Root and rhizome	<i>Saposhnikovia divaricata</i>	√	
JCS093	Fangji	Root and rhizome	<i>Stephania tetrandra</i>	√	
JCS052	Gancao	Root and rhizome	<i>Glycyrrhiza uralensis</i>	√	
JCS098	Ganjiang	Root and rhizome	<i>Zingiber officinale</i>		√
JCS099	Gaoliangjiang	Root and rhizome	<i>Alpinia officinarum</i>	√	
JCS001	Hongjingtian	Root and rhizome	<i>Rhodiola crenulata</i>	√	
JCS115	Huzhang	Root and rhizome	<i>Polygonum cuspidatum</i>		√
JCS117	Huainiuxi	Root and rhizome	<i>Achyranthes bidentata</i>	√	
JCS118	Huangqi	Root and rhizome	<i>Astragalus membranaceus</i>	√	
JCS006	Huangqin	Root and rhizome	<i>Scutellaria baicalensis</i>	√	
JCS139	Loulu	Root and rhizome	<i>Rhaponticum uniflorum</i>	√	√
JCS147	Maozhuacao	Root and rhizome	<i>Ranunculus ternatus</i>	√	
JCS026	Muxiang	Root and rhizome	<i>Aucklandia lappa</i>	√	
JCS025	Oujie	Root and rhizome	<i>Nelumbo nucifera</i>	√	
JCS156	Qianghuo	Root and rhizome	<i>Notopterygium incisum</i>	√	
JCS170	Shanyao	Root and rhizome	<i>Dioscorea opposita</i>		√
JCS173	Shegan	Root and rhizome	<i>Belamcanda chinensis</i>	√	
JCS174	Shengma	Root and rhizome	<i>Cimicifuga dahurica</i>	√	
JCS029	Shuifeiji	Root and rhizome	<i>Silybum marianum</i>	√	
JCS183	Tiankuizi	Root and rhizome	<i>Semiaquilegia adoxoides</i>	√	
JCS041	Tufuling	Root and rhizome	<i>Smilax glabra</i>		√
JCS194	Xiebai	Root and rhizome	<i>Allium macrostemon</i>	√	
JCS195	Xuchangqing	Root and rhizome	<i>Cynanchum paniculatum</i>	√	
JCS035	Xuduan	Root and rhizome	<i>Dipsacus asper</i>	√	
JCS202	Yinchaihu	Root and rhizome	<i>Stellaria dichotoma var. lanceolata</i>	√	
JCS038	Zexie	Root and rhizome	<i>Alisma plantago-aquatica</i>	√	
JCS032	Zhebeimu	Root and rhizome	<i>Fritillaria thunbergii</i>	√	
JCS036	Zhimu	Root and rhizome	<i>Anemarrhena asphodeloides</i>	√	
JCS211	Zicao	Root and rhizome	<i>Arnebia euchroma</i>	√	
JCS079	Ziwan	Root and rhizome	<i>Aster tataricus</i>	√	
JCS062	Baizhi	Root and rhizome	<i>Angelica dahurica</i>	√	
			<i>Angelica dahurica var. formosana</i>		
JCS018	Baihe	Root and rhizome	<i>Lilium pumilum</i>	√	
			<i>Lilium lancifolium</i>		
			<i>Ligusticum chuanxiong</i>		
JCS078	Chuanxiong	Root and rhizome	<i>Ligusticum sinense</i>		√
			<i>Ligusticum jeholense</i>		
			<i>Ligusticum acuminatum</i>		
			MI: <i>Ligusticum chuanxiong</i>		
JCS084	Dangshen	Root and rhizome	<i>Codonopsis tangshen</i>	√	
			<i>Codonopsis pilosula var. modesta</i>		
			<i>Codonopsis pilosula</i>		
JCS100	Gaoben	Root and rhizome	<i>Ligusticum sinense</i>	√	
			<i>Ligusticum jeholense</i>		
JCS051	Qianhu	Root and rhizome	<i>Peucedanum rubricaulle</i>	√	
			<i>Ligusticum involucratum</i>		
			<i>Ligusticum daucoides</i>		

Continued

Sample No.	Lable name	Medicinal part	Nearest match	ITS2	<i>psbA-trnH</i>
JCS162	Quanshen	Root and rhizome	<i>Bistorta vivipara</i>	√	
JCS054	Tianhuafen	Root and rhizome	<i>Trichosanthes rosthornii</i> <i>Trichosanthes kirilowii</i>	√	
JCS209	Zhigancao	Root and rhizome	<i>Glycyrrhiza uralensis</i> <i>Glycyrrhiza eurycarpa</i> <i>Glycyrrhiza aspera</i> MI: unable to identify	√	
JCS028	Baiqian	Root and rhizome	MI: <i>Cynanchum glaucescens</i>	/	/
JCS063	Baibu	Root and rhizome	MI: <i>Stemona tuberosa</i>	/	/
JCS027	Caowu	Root and rhizome	MI: unable to identify	/	/
JCS022	Chuanwu	Root and rhizome	MI: unable to identify	/	/
JCS039	Dancao	Root and rhizome	MI: <i>Gentiana rigescens</i>	/	/
JCS089	Ezhu	Root and rhizome	MI: <i>Curcuma phaeocaulis</i> <i>Curcuma kwangsiensis</i> <i>Curcuma wenyujin</i>	/	/
JCS097	Gansong	Root and rhizome	MI: <i>Nardostachys jatamansi</i>	/	/
JCS101	Gouji	Root and rhizome	MI: <i>Cibotium barometz</i>	/	/
JCS111	Hongshen	Root and rhizome	MI: <i>Panax ginseng</i>	/	/
JCS114	Huhuaglian	Root and rhizome	MI: <i>Picrorhiza scrophulariiflora</i>	/	/
JCS124	Jianghuang	Root and rhizome	MI: <i>Curcuma longa</i>	/	/
JCS030	Mingdangshen	Root and rhizome	MI: unable to identify	/	/
JCS153	Paojiang	Root and rhizome	MI: <i>Zingiber officinale</i>	/	/
JCS155	Qiannianjian	Root and rhizome	MI: <i>Homalomena occulta</i>	/	/
JCS157	Qinjiao	Root and rhizome	MI: <i>Gentiana dahurica</i>	/	/
JCS169	Shannai	Root and rhizome	MI: <i>Kaempferia galanga</i>	/	/
JCS179	Shouwu	Root and rhizome	MI: <i>Fallopia aubertii</i>	/	/
JCS055	Tianma	Root and rhizome	MI: <i>Gastrodia elata</i>	/	/
JCS190	Xianmao	Root and rhizome	MI: <i>Curculigo orchiooides</i>	/	/
JCS205	Yujin	Root and rhizome	MI: <i>Curcuma wenyujin</i> <i>Curcuma longa</i> <i>Curcuma kwangsiensis</i> <i>Curcuma phaeocaulis</i>	/	/
JCS045	Yuanhu	Root and rhizome	MI: <i>Corydalis yanhusuo</i>	/	/
JCS210	Chonglou	Root and rhizome	MI: <i>Paris polyphylla</i> var. <i>yunnanensis</i> <i>Paris polyphylla</i> var. <i>chinensis</i>	/	/
JCS064	Baiziren	Fruit and seed	<i>Platycladus orientalis</i>	√	
JCS069	Biandou	Fruit and seed	<i>Dolichos lablab</i>	√	
JCS021	Buguzhi	Fruit and seed	<i>Psoralea corylifolia</i>	√	
JCS071	Caokou	Fruit and seed	<i>Alpinia hainanensis</i>	√	
JCS085	Difuzi	Fruit and seed	<i>Kochia scoparia</i>	√	
JCS087	Dongguapi	Fruit and seed	<i>Benincasa hispida</i>	√	
JCS046	Gouqizi	Fruit and seed	<i>Lycium barbarum</i>	√	
JCS110	Heidou	Fruit and seed	<i>Glycine max</i>	√	
JCS132	Lianqiao	Fruit and seed	<i>Forsythia suspensa</i>	√	
JCS134	Lianrou	Fruit and seed	<i>Nelumbo nucifera</i>	√	
JCS136	Lianzixin	Fruit and seed	<i>Nelumbo nucifera</i>	√	
JCS151	Niubangzi	Fruit and seed	<i>Arctium lappa</i>	√	
JCS159	Qingguo	Fruit and seed	<i>Canarium album</i>	√	
JCS168	Shayuanzi	Fruit and seed	<i>Astragalus complanatus</i>	√	
JCS172	Shechuangzi	Fruit and seed	<i>Cnidium monnieri</i>	√	
JCS176	Shengmaiya	Fruit and seed	<i>Hordeum vulgare</i>	√	
JCS177	Shengzaoren	Fruit and seed	<i>Ziziphus jujuba</i> var. <i>spinosa</i>	√	
JCS044	Suzi	Fruit and seed	<i>Perilla frutescens</i>	√	
JCS212	Suzi	Fruit and seed	<i>Perilla frutescens</i>	√	
JCS184	Tusizi	Fruit and seed	<i>Cuscuta australis</i>	√	√
JCS186	Wuzhuyu	Fruit and seed	<i>Euodia rutaecarpa</i>	√	
JCS007	Xiakucao	Fruit and seed	<i>Prunella vulgaris</i>	√	
JCS192	Xiaohuixiang	Fruit and seed	<i>Foeniculum vulgare</i>	√	
JCS197	Yadanzi	Fruit and seed	<i>Brucea javanica</i>	√	
JCS206	Yuzhizi	Fruit and seed	<i>Akebia quinata</i>	√	

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Sample No.	Lable name	Medicinal part	Nearest match	ITS2	<i>psbA-trnH</i>	
JCS208	Zhizi	Fruit and seed	<i>Gardenia jasminoides</i>	√		
JCS074	Cheqianzi	Fruit and seed	<i>Plantago asiatica</i> <i>Plantago major</i> MI: <i>Plantago asiatica</i>	√		
JCS076	Chixiaodou	Fruit and seed	<i>Vigna umbeuata</i> <i>Vigna angularis</i>	√		
JCS077	Chongweizi	Fruit and seed	<i>Leonurus sibiricus</i>	√		
JCS094	Foshou	Fruit and seed	<i>Citrus medica</i> var. <i>sarcodactylis</i> and multiple species in the same genus MI: <i>Citrus medica</i> var. <i>sarcodactylis</i>	√		
JCS102	Gualou	Fruit and seed	<i>Trichosanthes rosthornii</i> <i>Trichosanthes kirilowii</i>	√		
JCS103	Gualoupi	Fruit and seed	<i>Trichosanthes rosthornii</i> <i>Trichosanthes kirilowii</i>	√		
JCS116	Huajuhong	Fruit and seed	<i>Citrus grandis</i> 'Tomentosa' and multiple species in the same genus MI: <i>Citrus grandis</i> 'Tomentosa'	√		
JCS166	Sangshen	Fruit and seed	<i>Morus alba</i> and multiple species in the same genus MI: unable to identify	√		
JCS178	Shengzhiqiao	Fruit and seed	<i>Citrus aurantium</i> and multiple species in the same genus MI: <i>Citrus aurantium</i>	√		
JCS057	Baiguo	Fruit and seed	MI: <i>Ginkgo biloba</i>	/	/	
JCS070	Cangerzi	Fruit and seed	MI: <i>Xanthium sibiricum</i>	/	/	
JCS050	Dafupi	Fruit and seed	MI: <i>Areca catechu</i>	/	/	
JCS082	Dandouchi	Fruit and seed	MI: <i>Glycine max</i>	/	/	
JCS096	Fupenzi	Fruit and seed	MI: <i>Rubus chingii</i>	/	/	
JCS107	Hezi	Fruit and seed	MI: <i>Terminalia chebula</i> <i>Terminalia chebula</i> var. <i>tomentella</i>	/	/	
JCS129	Juhe	Fruit and seed	MI: unable to identify	/	/	
JCS130	Juemingzi	Fruit and seed	MI: <i>Cassia tora</i>	/	/	
JCS138	Longyanrou	Fruit and seed	MI: <i>Dimocarpus longan</i>	/	/	
JCS141	Lulutong	Fruit and seed	MI: <i>Liquidambar formosana</i>	/	/	
JCS146	Manjingzi	Fruit and seed	MI: <i>Vitex trifolia</i> <i>Vitex trifolia</i> var. <i>simplicifolia</i>	/	/	
JCS150	Mugua	Fruit and seed	MI: unable to identify	/	/	
JCS152	Nvzhenzi	Fruit and seed	MI: <i>Ligustrum lucidum</i>	/	/	
JCS161	Qingpi	Fruit and seed	MI: unable to identify	/	/	
JCS171	Shanzhuyu	Fruit and seed	MI: <i>Cornus officinalis</i>	/	/	
JCS187	Xiqingguo	Fruit and seed	MI: <i>Terminalia chebula</i>	/	/	
JCS200	Yizhiren	Fruit and seed	MI: <i>Alpinia oxyphylla</i>	/	/	
JCS058	Baihuasheshecao	Herb	<i>Oldenlandia diffusa</i>	√		
JCS019	Banbianlian	Herb	<i>Lobelia chinensis</i>	√		
JCS004	Banzhilian	Herb	<i>Scutellaria barbata</i>	√		
JCS065	Bohe	Herb	<i>Mentha haplocalyx</i>	√		
JCS067	Beiliujinu	Herb	<i>Siphonostegia chinensis</i>	√		
JCS068	Bianxu	Herb	<i>Polygonum aviculare</i>	√		
JCS003	Chuanxinlian	Herb	<i>Andrographis paniculata</i>	√		
JCS048	Daji	Herb	<i>Cirsium japonicum</i>	√		
JCS005	Diding	Herb	<i>Corydalis bungeana</i>	√		
JCS090	Ebushicao	Herb	<i>Centipeda minima</i>	√		
JCS095	Fuping	Herb	<i>Spirodela polyrrhiza</i>		√	
JCS104	Guanghuoxiang	Herb	<i>Desmodium styracifolium</i>	√		
JCS119	Huoxiang	Herb	<i>Pogostemon cablin</i>	√		
JCS123	Jixuecao	Herb	<i>Centella asiatica</i>	√		
JCS127	Jingjie	Herb	<i>Schizonepeta tenuifolia</i>	√		
JCS131	Laoguancao	Herb	<i>Geranium carolinianum</i>	√		
JCS031	Mabiancao	Herb	<i>Verbena officinalis</i>	√		
JCS145	Machixian	Herb	<i>Portulaca oleracea</i>	√		
JCS148	Mohanlian	Herb	<i>Eclipta prostrata</i>	√		
JCS002	Pugongying	Herb	<i>Taraxacum mongolicum</i>	√		
JCS160	Qinghao	Herb	<i>Artemisia annua</i>	√		
JCS009	Qumai	Herb	<i>Dianthus chinensis</i>	√		

Continued

Sample No.	Lable name	Medicinal part	Nearest match	ITS2	psbA-trnH
JCS015	Shenjinciao	Herb	<i>Lycopodium japonicum</i>		√
JCS181	Sugeng	Herb	<i>Perilla frutescens</i>	√	
JCS182	Suoyang	Herb	<i>Cynomorium songaricum</i>	√	
JCS049	Weilingcai	Herb	<i>Potentilla chinensis</i>	√	
JCS189	Xianhecao	Herb	<i>Agrimonia pilosa</i>	√	
JCS193	Xiaoji	Herb	<i>Cirsium setosum</i>	√	
JCS020	Yazhicao	Herb	<i>Commelina communis</i>	√	
JCS199	Yimucao	Herb	<i>Leonurus japonicus</i>	√	
JCS034	Yinyanghuo	Herb	<i>Epimedium koreanum</i>	√	
JCS204	Yuxingcao	Herb	<i>Houttuynia cordata</i>	√	
JCS047	Zelan	Herb	<i>Lycopus lucidus</i>	√	
JCS086	Dijincao	Herb	<i>Euphorbia humifusa</i>	√	
			<i>Polygonum aviculare</i>		
JCS120	Jiguciao	Herb	<i>Abrus cantoniensis</i>	√	
			<i>Abrus mollis</i>		
			MI: above two species		
JCS008	Jinqiancao	Herb	<i>Desmodium styracifolium</i>	√	
JCS128	Juju	Herb	<i>Sonchus arvensis</i>	√	
			<i>Lactuca indica</i>		
			MI: adulterant		
JCS140	Luxiancao	Herb	<i>Pyrola rotundifolia</i>	√	
			<i>Pyrola incarnata</i>		
			<i>Pyrola grandiflora</i>		
JCS154	Peilan	Herb	<i>Eupatorium fortune</i>	√	
			<i>Eupatorium lindleyanum</i>		
			MI: above two species		
JCS188	Xixiancao	Herb	<i>Siegesbeckia orientalis</i>	√	
			<i>Xanthium sibiricum</i>		
JCS201	Yinchen	Herb	<i>Artemisia scoparia</i>	√	
			<i>Artemisia capillaris</i>		
JCS125	Jinfeicao	Herb	MI: adulterant	/	/
JCS163	Roucongong	Herb	MI: <i>Cistanche deserticola</i>	/	/
			<i>Cistanche tubulosa</i>		
JCS185	Wasong	Herb	MI: <i>Orostachys fimbriata</i>	/	/
JCS191	Xiangru	Herb	MI: unable to identify	/	/
JCS108	Hehuanhua	Flower	<i>Albizia julibrissin</i>	√	
JCS053	Huaihua	Flower	<i>Sophora japonica</i>		√
JCS121	Jiguanhua	Flower	<i>Celosia cristata</i>	√	
JCS126	Jinyinhua	Flower	<i>Lonicera japonica</i>	√	
JCS042	Jingjiesui	Flower	<i>Schizonepeta tenuifolia</i>	√	
JCS011	Kuandonghua	Flower	<i>Tussilago farfara</i>	√	
JCS135	Lianxu	Flower	<i>Nelumbo nucifera</i>	√	
JCS012	Meihua	Flower	<i>Prunus mume</i>	√	
JCS016	Juhua	Flower	<i>Chrysanthemum morifolium</i> and multiple species in the same genus	√	
			MI: <i>Chrysanthemum morifolium</i>		
JCS013	Puhuang	Flower	<i>Typha</i> sp.	√	
JCS196	Xuanfuhua	Flower	<i>Inula linariifolia</i>	√	
JCS198	Yejuhua	Flower	<i>Chrysanthemum indicum</i>	√	
			<i>Chrysanthemum vestitum</i>		
			MI: <i>Chrysanthemum indicum</i>		
JCS113	Houpohua	Flower	MI: <i>Magnolia officinalis</i>	/	/
			<i>Magnolia officinalis</i> var. <i>biloba</i>		
JCS133	Lianfang	Flower	MI: <i>Nelumbo nucifera</i>	/	/
JCS014	Lingxiaohua	Flower	MI: <i>Campsis radicans</i>	/	/
JCS056	Aiye	Leaf	<i>Artemisia argyi</i>	√	
JCS083	Danzhuye	Leaf	<i>Lophatherum gracile</i>		√
JCS109	Heye	Leaf	<i>Nelumbo nucifera</i>	√	
JCS137	Liaodaqingye	Leaf	<i>Polygonum tinctorium</i>	√	
JCS142	Luobumaye	Leaf	<i>Apocynum venetum</i>	√	
JCS149	Mufurongye	Leaf	<i>Hibiscus mutabilis</i>	√	

Sample No.	Lable name	Medicinal part	Nearest match	Continued	
				ITS2	<i>psbA-trnH</i>
JCS017	Pipaye	Leaf	<i>Eriobotrya japonica</i>	√	
JCS175	Shengcebai	Leaf	<i>Platycladus orientalis</i>	√	
JCS040	Suye	Leaf	<i>Perilla frutescens</i>	√	
JCS203	Yinxingye	Leaf	<i>Ginkgo biloba</i>	√	
JCS167	Sangye	Leaf	<i>Morus alba</i> and multiple species in the same genus MI: <i>Morus alba</i>	√	
JCS080	Chunpi	Cortex	<i>Ailanthus altissima</i>	√	
JCS112	Houpo	Cortex	<i>Magnolia officinalis</i>		√
JCS081	Danpi	Cortex	<i>Paeonia suffruticosa</i> <i>Paeonia ostii</i> <i>Paeonia rockii</i> subsp. <i>rockii</i> MI: <i>Paeonia suffruticosa</i> <i>Paeonia ostii</i>	√	
JCS165	Sangbaipi	Cortex	<i>Morus alba</i> and multiple species in the same genus MI: unable to identify	√	
JCS158	Qinpi	Cortex	MI: <i>Fraxinus rhynchophylla</i> <i>Fraxinus chinensis</i> <i>Fraxinus szaboana</i> <i>Fraxinus stylosa</i>	/	/
JCS023	Sangpi	Cortex	MI: unable to identify	/	/
JCS105	Guizhi	Caulis	<i>Cinnamomum cassia</i>		√
JCS122	Jixueteng	Caulis	<i>Spatholobus suberectus</i>	√	
JCS143	Luoshiteng	Caulis	<i>Trachelospermum jasminoides</i>	√	
JCS180	Shouwuteng	Caulis	<i>Fallopia multiflora</i>	√	
JCS106	Haijinsha	Other	<i>Lygodium japonicum</i>		√
JCS207	Zaojiaoci	Other	<i>Gleditsia sinensis</i>	√	
JCS033	Haizao	Other	<i>Sargassum fusiforme</i>	√	
JCS091	Ercha	Other	MI: unable to identify	/	/
JCS144	Mabo	Other	MI: <i>Lasiosphaera fenlzii</i> <i>Calvatia gigantea</i> <i>Calvatia lilacina</i>	/	/
JCS164	Ruxiang	Other	MI: unable to identify	/	/

Table 2 Primers and PCR conditions used in the experiment

Locus	Primer name	Sequences (5'-3')	PCR conditions
ITS2	S2F	ATGCGATACTTGGTGTGAAT	94 °C 5 min
	S3R	GACGCTTCTCCAGACTACAAT	94 °C 30 s, 56 °C 30 s, 72 °C 45 s, 40 cycles 72 °C 10 min
<i>psbA-trnH</i>	fwd	GTTATGCATGAACGTAATGCTC	95 °C 4 min
	rev	CGCGCATGGTGGATTCAATCC	94 °C 30 s, 55 °C 1 min, 72 °C 1 min, 35 cycles 72 °C 10 min

结果

1 市售中药饮片DNA条形码序列获取情况

本研究共涉及中药饮片样本212份,经DNA提取及PCR扩增后,共有161份样本成功获得PCR扩增产物,占收集中药饮片样本量的75.9%,其中147份样本扩增ITS2序列,12份样本扩增*psbA-trnH*序列,2份样本扩增ITS2和*psbA-trnH*序列;其余50份样本基因组DNA降解,无法获得扩增产物;此外,1份样本为树脂(乳香),不含基因组DNA,故未进行DNA提取实验。

以紫草、栀子、薄荷、鸡冠花、红参、苍耳子、莲房等为例展示不同入药部位中药饮片基因组DNA及ITS2序列PCR扩增产物电泳结果(图1)。PCR扩增产物经纯化后进行双向测序,测序结果经拼接后,获得ITS2序列149条,*psbA-trnH*序列14条,不同入药部位中药饮片样本获取DNA条形码序列数量见图2。

2 市售中药饮片DNA条形码鉴定结果

采用“中药材DNA条形码鉴定系统(<http://www.tcmbarcode.cn>)”对实验所得161份中药饮片样本的

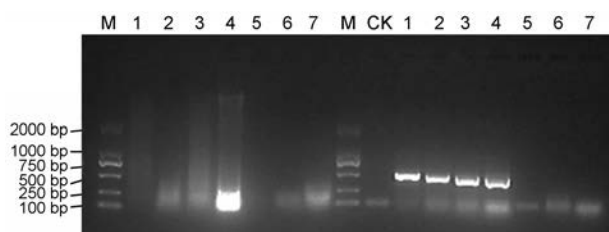


Figure 1 Genomic DNA and PCR amplification results of ITS2 sequence of the commercial decoction pieces (Left side: Genomic DNA; Right side: PCR products). M: DL2000, CK: Negative control. 1: Zicao, 2: Zhizi, 3: Bohe, 4: Jiguanhua, 5: Hongshen, 6: Cangerzi, 7: Lianfang

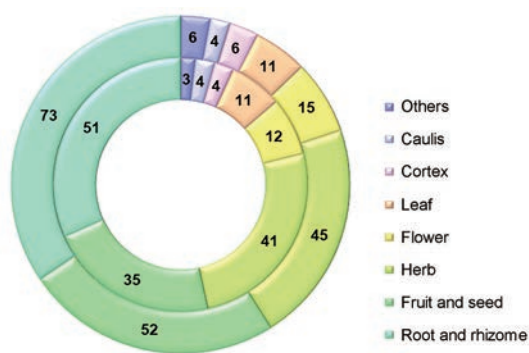


Figure 2 Sample size and the DNA barcoding sequence number of different medicinal parts of the decoction pieces. (Outer ring: Sample size; inner ring: DNA barcoding sequence number)

ITS2 序列或 (和) *psbA-trnH* 序列进行结果判定, 获得与该序列相似度最高的物种, 详细结果见表 1。结果显示, 161 份饮片样本中, 138 份样本为《中国药典》(2020 年版一部) 收载相应药材的基原物种, 占 85.7%; 14 份样本分析结果中包含《中国药典》收载相应药材的基原物种以及同属近缘物种, 分别为鸡骨草比对为广州相思子 *Abrus pulchellus* subsp. *cantoniensis*/毛鸡骨草 *Abrus mollis*, 野菊花比对为野菊 *Chrysanthemum indicum*/毛华菊 *Chrysanthemum vestitum*, 生枳壳、化橘红、佛手均比对为包含各自基原物种在内的同属多个物种, 佩兰比对为佩兰 *Eupatorium fortunei*/轮叶泽兰 *Eupatorium lindleyanum*, 炙甘草比对为甘草 *Glycyrrhiza uralensis*/粗毛甘草 *Glycyrrhiza aspera*/胀果甘草 *Glycyrrhiza eurycarpa*, 川芎比对为川芎 *Ligusticum chuanxiong*/藁本 *Ligusticum sinense*/辽藁本 *Ligusticum jeholense*/尖叶藁本 *Ligusticum acuminatum*, 桑白皮、桑葚、桑叶均比对为桑 *Morus alba* 及其同属多个物种, 丹皮比对为牡丹 *Paeonia suffruticosa*/凤丹 *Paeonia ostii*/紫斑牡丹 *Paeonia rockii* subsp. *rockii*, 车前子比对为车前 *Plantago asiatica*/大车前 *Plantago major*, 菊花比对为菊 *Chrysanthemum morifolium* 及其同属多个物种; 7 份样

本比对结果显示为混伪品, 分别为拳参比对为珠芽蓼 *Bistorta vivipara*、金钱草比对为广金钱草 *Desmodium styracifolium* (图 3)、旋覆花比对为条叶旋覆花 *Inula linariifolia*、茺蔚子比对为细叶益母草 *Leonurus sibiricus*、前胡比对为红前胡 *Peucedanum rubricaulis*/多苞藁本 *Ligusticum involucreatum*/羽苞藁本 *Ligusticum daucoides*、鹿衔草比对为圆叶鹿蹄草 *Pyrola rotundifolia*/红花鹿蹄草 *Pyrola incarnata*/*Pyrola grandiflora*, 菊苣比对为苣荬菜 *Sonchus arvensis*/翅果菊 *Lactuca indica*; 2 份样本比对结果显示样本中存在掺伪现象, 分别为地锦草和豨莶草, 其中地锦草饮片中检出蓊蓄 *Polygonum aviculare*, 豨莶草饮片中检出苍耳 *Xanthium sibiricum*。

3 性状鉴定结果

上述结果显示, 本研究共有 50 份样本基因组 DNA 降解, 无法获得扩增产物, 1 份样本为树脂类药材, 不含有基因组 DNA; 14 份样本分析结果中包含《中国药典》收载相应药材的基原物种以及同属近缘物种。为进一步确定以上 65 份饮片样本基原, 对其进行性状鉴定, 结果如下: ① 50 份基因组 DNA 降解的样本, 38 份鉴定为《中国药典》收载相应药材的基原物种, 3 份样本鉴定为混伪品, 分别为百部鉴定为大百部 *Stemona tuberosa*、首乌鉴定为木藤蓼 *Fallopia aubertii*、金沸草鉴定为伪品 (不能确定伪品物种), 9 份样本缺少鉴别特征, 不能确定基原, 分别为川乌、桑皮、草乌、明党参、儿茶、橘核、木瓜、青皮、香薷; ② 1 份树脂样本 (乳香) 缺乏鉴别特征, 不能确定基原; ③ 14 份分析结果包含同属近缘物种的样本, 8 份样本鉴定为正品, 3 份样本中掺有伪品, 分别为丹皮、鸡骨草、佩兰, 3 份样本缺少鉴别特征, 不能确定基原, 分别为桑白皮、桑葚、炙甘草。详细结果见表 1。

综合 DNA 条形码鉴定及性状鉴定结果, 本研究涉及的 212 份样本中, 共有 184 份为正品, 占总样本量 86.8%; 15 份存在混伪/掺伪现象; 13 份缺少鉴别特征, 无法确定基原。

讨论

中药饮片是指在中医药理论指导下, 可直接用于调配或制剂的中药材及其加工炮制品。中药饮片包括部分经产地加工的中药切片 (包括切段、块、瓣), 原形药材饮片以及经过切制 (在产地加工的基础上)、炮炙的饮片。中药饮片既能直接配方使用, 如制丸剂、散剂, 又可以继续加工, 如煎制汤剂、熬制膏方, 亦或用于中成药生产, 其真伪优劣直接关乎患者生命健康安全。中药饮片在炮制加工过程中会损失部分性状鉴别特征, 且较之原药材而言更易发生掺伪现象, 使得中药饮

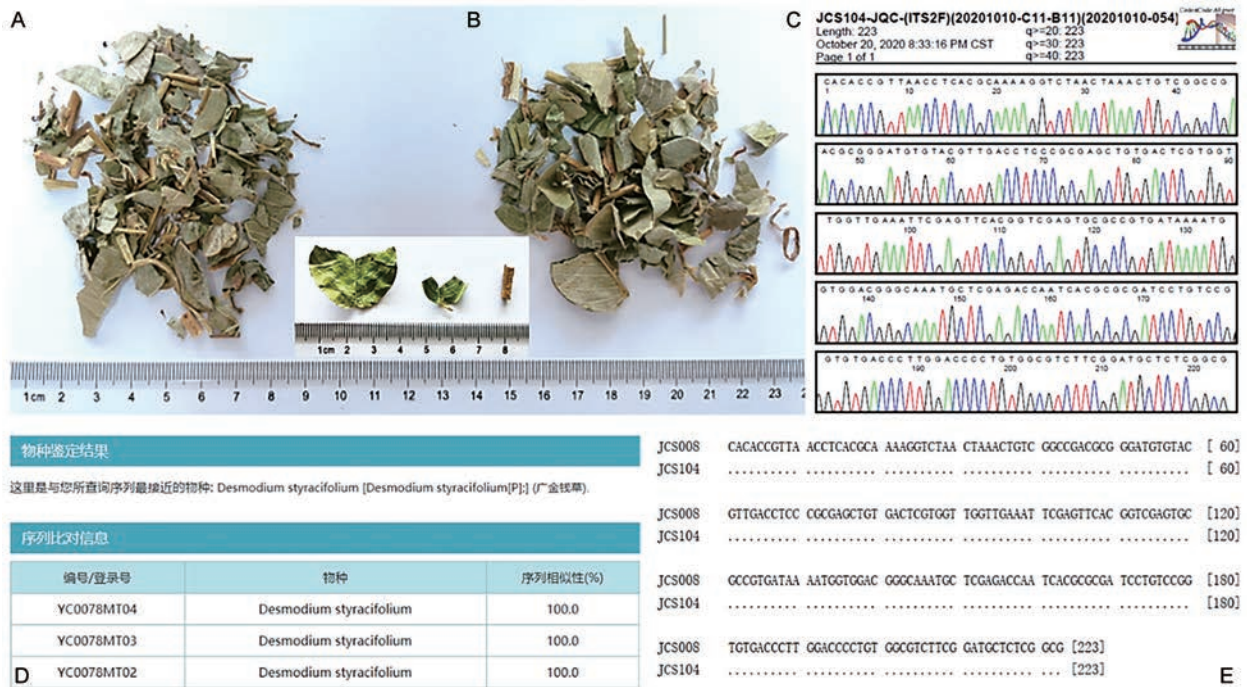


Figure 3 Morphological and DNA barcoding identification of Jinqiancao and Guangjinqiancao. A: Jinqiancao; B: Guangjinqiancao; C: Sequencing result of Guangjinqiancao; D: DNA barcoding identification with "DNA barcoding system for identifying herbal medicine"; E: ITS2 Sequence alignment of Jinqiancao and Guangjinqiancao

片真伪鉴定难度加大。本研究涉及的212份样本均为普通民众在药店中极易获取的中药饮片, DNA条形码分子鉴定结果显示138份样本为《中国药典》(2020年版一部) 记载相应药材的基原物种, 占获得序列样本数的85.7%; 14份样本分析结果中包含《中国药典》记载相应药材的基原物种以及同属近缘物种, 占获得序列样本数的8.7%; 另有9份样本存在掺伪/混伪现象, 占获得序列样本数的5.6%。以上结果表明, 中药材DNA条形码分子鉴定技术基本适用于市售中药饮片的基原物种鉴定工作, 能够有效检出市售中药饮片的掺伪/混伪现象, 值得在中药饮片加工生产企业及药品监督检验管理机构推广。

另有50份样本(占总样本量23.58%) 因炮制加工程度较高, 如蒸/煮透心、沙烫、炒焦、炒黄、炒炭等, 基因组DNA降解严重, 导致无法获取DNA条形码序列。该结果提示, 中药材DNA条形码分子鉴定法的常规标准操作流程对于炮制后的中药饮片适用性不佳, 为解决此问题, 本研究对上述样本进行性状鉴定, 38份鉴定为正品, 3份样本鉴定为混伪品, 显示性状鉴别方法在中药材及饮片基原鉴定工作中的重要性, 但该方法要求实施者具备扎实的中药鉴定学专业知识及丰富的饮片鉴别实践经验, 非专业人员掌握此方法难度较大。此外, 还可采用高通量测序技术对基因组DNA直接测

序后通过生物信息学分析筛选并组装出DNA条形码序列^[14], 或有针对性地ITS2序列或*psbA-trnH*序列内部设计引物扩增其特异性短序列作为分子身份证^[15,16], 亦或设计TaqMan探针^[17]等, 以此实现对基因组DNA降解严重的中药饮片的分子鉴定。

本研究发现, 饮片商品标签名称存在与实际物种不符, 或名称易混淆等现象。如商品标签名称为“地丁”的饮片, 单从名称来看无法确定是“紫花地丁”还是“苦地丁”, 但该份饮片样本中可见扁长椭圆形蒴果, 呈荚果状, 内含扁心形黑色具光泽的种子, 与《中国药典》(2020年版一部) 记载“苦地丁”饮片的果实及种子性状特征描述一致, 其基原物种为地丁草 *Corydalis bungeana*, 这与DNA条形码鉴定结果一致, 因此其商品标签应标注为“苦地丁”, 以免药店人员抓错药, 类似的情况还有“菖蒲”应明确标注为“石菖蒲”。另有部分饮片名称汉字有误, 如“紫苑”应写作“紫菀”, “山柰”应写作“山柝”, “化桔红”应写作“化橘红”等, 也值得从业人员重视。此外, 按《中国药典》(2020年版一部) 规定, 中药金钱草基原物种为报春花科聚花过路黄 *Christina lysimachiae*, 广金钱草基原物种为豆科广金钱草 *Desmodium styracifolium*, 二者来源于不同科。但本研究涉及的商品标签名为“金钱草”和“广金钱草”的两种饮片外观性状极为相似、难以区分(图3), 两

个样本实验所得 ITS2 序列测序结果均质量极佳, 序列无变异位点, 经“中药材 DNA 条形码分子鉴定系统”分析证实两份饮片基原物种均为广金钱草 *Desmodium styracifolium*。发生该现象的潜在原因有两点: ① 因“金钱草”资源量及产量低于“广金钱草”, 且价格高于“广金钱草”, 饮片生产者有意用“广金钱草”替代“金钱草”出售, 从中牟利, 属于人为混伪; ② 药店商品标签标识不清, 或从业人员专业知识水平有限, 未能明确区分两种饮片, 抓药时误将两种饮片当做同一种饮片出售给消费者。以上实例提示, 药店在出售饮片时应当依照《中国药典》规范标注饮片名称, 以免造成混淆, 影响临床用药安全。

又如中药“泽泻”, 《中国药典》(2015年版一部) 记载其为泽泻科植物泽泻 *Alisma orientale* (Sam.) Juzep. 的干燥块茎, 然而依据《中国植物志》记载, 中文名“泽泻”对应的物种拉丁名应当为 *Alisma plantago-aquatica* L., 而拉丁名 *Alisma orientale* (Samuel.) Juz. 对应的物种中文名应当为“东方泽泻”, 上述两个物种外观性状极为相似、难以区分, 且市场上均有销售, 属于因名称混淆导致的中药材混伪现象。文献报道显示 DNA 条形码技术能够有效鉴别上述两个物种, 虽然二者 ITS2 序列仅存在 1 个 SNP 位点, 但能够作为市售泽泻药材基原物种的鉴别特征^[18], 显示 DNA 条形码分子鉴定技术的准确性。目前, 现行版《中国药典》(2020年版一部) 已对中药“泽泻”的基原物种进行修订, 指明该药材来源于泽泻科东方泽泻 *Alisma orientale* (Sam.) Juzep. 或泽泻 *Alisma plantago-aquatica* Linn., 而本研究涉及的饮片“泽泻”, 经 DNA 条形码鉴定为泽泻 *Alisma plantago-aquatica*, 依照现行版《中国药典》, 应视为正品。

对于饮片“地锦草”和“豨莶草”, 在实验过程中发现所购饮片中存在性状明显不同的样本, 因此分别取样并进行 DNA 条形码分子鉴定, 结果显示, 饮片“地锦草”中检出“蒺藜”, “豨莶草”中检出“苍耳”, 上述药材或饮片因外观性状差异明显, 可直接区分, 故不属于混伪品, 发生此现象的潜在原因可能为: ① 药材或饮片生产加工过程中, 因晾晒、切制或储藏位置相近, 工人在操作过程中无意识混入; ② 不同药材及饮片混用生产线, 导致残存在机器中的前一种药材的碎片被混入后续加工的其他药材。该案例提示中药材及饮片生产加工企业应规范生产操作流程, 制定有效的监管措施, 避免此类情况发生。在新版《中华人民共和国药品管理法》实施后, 虽然取消了 GMP 认证, 但也意味着国家对药品监管措施更加严格, 中药全产业链都应当从根本上重视原材料的准确性, DNA 条形码分子鉴定技术

在中药全产业链各环节发挥其重要作用^[19,20], 有助于保证中药产业健康发展, 推动中药走向世界。

中医药作为凝聚中华民族传统哲学智慧的宝库, 为人类健康做出的贡献举世瞩目。自抗疟药青蒿素的发现获得 2015 年诺贝尔生理学或医学奖以来, 公众对中医药的关注程度日渐加深, 本草基因组学为传统中药和现代生命科学架起沟通的桥梁^[21], 大量西医学或生命科学领域的研究人员开始将目光投向中药, 并从不同角度对中药药效的作用机制展开探讨。例如, 研究人员在金银花水煎液中发现一种 miRNA——MIR2911, 它能够显著抑制 H1N1、H5N1、H7N9 病毒复制, 且显著降低感染 H5N1 病毒小鼠的死亡率, 这是中药中发现的首个直接作用于流感病毒 (IAVs) 的活性成分^[22]。此外, 最新研究表明, MIR2911 能够显著抑制 SARS-CoV-2 病毒 (新型冠状病毒) 复制, 且在临床上能加速患者转阴^[23]。蒋澄宇研究员团队发现红景天中的 miRNA HJT-sRNA-m7 可有效降低肺纤维化标志基因及蛋白表达水平, 创新性提出中药材 miRNA 可作为口服药物的治疗策略^[24]。以上研究均为中药创新药物研发提供新思路。但因非中医药领域的研究人员对中药鉴定学专业知识了解有限, 在选定研究对象后, 去市场上直接购买药材或饮片时, 无法准确判断所购药材或饮片是否为《中国药典》记载的正品基原物种, 存在买到混伪品或掺伪品的可能性。如若开展实验前未对所购样品进行基原鉴定, 可能获得与预期不一致的实验结果, 影响研究进度, 亦会造成科研经费及资源浪费。因此, 建议在实验前务必对实验材料进行基原鉴定。中药材 DNA 条形码分子鉴定技术操作简便、快捷, 非专业人员也能够在短时间内掌握该技术, 因而值得向其他学科研究人员推广该技术, 辅助其研究工作顺利进行, 同时也有助于促进中药学研究的跨学科交流, 共同挖掘中药作用靶点和药效机制, 推动个性化精准医疗及“精准药物研发”。

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利益冲突: 本文的研究内容无任何利益冲突。

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