INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.12.10.2021.11(49)

A DNA FINGER PRINTING TECHNIQUE ANALYSIS USED IN HERBAL FORMULATIONS

Kundla. Jaya Lakshmi[1]*, K. Vinodkumar[1] and K. Sai Lakshmi[2]

[1]Department of pharmaceutical analysis, Raghavendra Institute of Pharmaceutical Education and Research [RIPER] - Autonomous, K.R.Palli cross, Anantapur-515721, Andhra Pradesh, India.

[2]Department of Pharmaceutical Analysis, Jawaharlal Nehru Technological University (OTPRI), Anantapur-515721, Andhra Pradesh, India.

ABSTRACT

Ancient Indian medicinal herbs played an important role in environmental protection and preventing illness worldwide in olden history. Medicinal herbs consist primarily of pieces of fresh plant extracts, oils, and gums. A combination of herbs, especially in developing countries, forms an essential part of the medicinal herbs system. In the past, present, and future, DNA fingerprinting in plants by botany is the experience of authorized DNA fingerprints. Approximately 75% of the world's population is based on this medicine process, and we have a pharmacy demand of $62 billion and are predicted to rise to $5 trillion by 2050. In this investigation, fingerprinting techniques, PCR stages, complex genotyping techniques, DNA-based herbal plant authentication techniques such as DNA techniques, DNA barcoding, real-time PCR, HRM analysis, and sophisticated DNA-based tools such as (HAD, LAMP, and NGS) have been used to authenticate fresh herbs and/or herbal products for the analysis of DNA strategies. However, if the products were mainly made during production from pills, powders, tablets, or dried varieties. In this study, the use of DNA approaches is summarized, while still providing relevant and accurate applications on herbal formulations.

Keywords: DNA fingerprinting, DNA barcoding, Herbal medicine, Finger printing technique.

1. INTRODUCTION

Since the historical era, herbal medicines are being implemented through both modern medicine and traditional medicine systems and are believed to be the oldest health care commodities. Ayurvedic medicinal herbs have a crucial role in preserving health and communicating with the disorder globally since past cultures. Because of Ayurvedic medicine & herbalist's success accomplish one way to develop and flourish, the global market sector approximately 60 billion annually, with a sales revenue on global markets of 3,500 crore rupees of herbal medicine.[1,2] Authenticity, purity, and protection are critical elements of standardization according to WHO standards, and authentication is the timeline stage in the assessment of traditional medicines. Truthfulness has to do with the verification that the content is genuine and corresponds to the right identity. Quality and Assurance of herbal ayurvedic drugs is the biggest difference between commercialization and excess supply protection. Traditional herbal plant species are frequently either inadvertently or intentionally substituted and/or adulterated with herbs from plants that are clinically similar or with tightly bound together substances from similar plants. Herbal consumption typically occurs as a result of ingredients that do not have different morphological features, materials and identical generic names, and the replacement of commercially useful materials for inexpensive herbs. Herbal remedies have been used as medicines for the treatment of various diseases since ancient times. Proper

authentication is essential to protect the adulteration of herbal plants with other plant materials. For the standardization of botanical preparations, some regulatory authorities and pharmacopeia propose a macroscopic, microscopic, and chemical assessment.[3, 4, and 5]

2. METHODOLOGY

2.1 DNA fingerprinting

Bar-code-like sensor - enabled by an individual's replication of genome DNA are DNA fingerprints. It is based on the molecular identity of a genetic organism. It focuses on the specific genotype of the method and the main gene, to identify the concentrations of a set of characteristic secondary metabolites in the herb. DNA Finger Printing was invented in 1984 at Leicester University by Prof. Alex Jeffery. It is the greatest contribution of plants and animals to the later growth of human genetics. Biological samples used for DNA fingerprinting: DNA samples of body tissue from Blood Hair Saliva Skin Consumer usage. The uses of DNA fingerprinting are the particular fingerprint as a precision, separating genuine drugs from replaced/adulterated drugs regardless of environmental factors Regardless of plant physiology.

Fig 1: Fingerprinting techniques

The standard approach for Genetic analysis in plants includes, first of all, isolation of DNA from plants and risk identification. By removing Within DNA and separating DNA from much other range of product, such as cell fragments, proteins, enzymes, or RNA, the cell wall, and cytoplasmic envelope, DNA from plant tissue is extracted without compromising the integrity of the DNA. Proactive maintenance and product quality of medicinal herbal products. [6, 7] most regulatory guidelines and pharmacopeia show microscopic and macroscopic examination and chemical testing of herbal products for quality control and standardization. Usually, molecular methods refer to bioactive metabolites, including chemical compounds, and other nucleotide-like macromolecules. For product quality and herbal medicine optimization as names, natural products have been used heavily.[8, 9]

2.2 DNA fingerprinting of the past, present, and future in plants

In DNA fingerprinting, a multitude of techniques has been developed, refined, and finally scrapped as new and more effective, and far more reliable methods have emerged. Despite some overlap between the life of various technologies, the past, current, and future stages, which coincide with major technological advances, can be classified.[2,3,4]

Several botanical disciplines depend on the ability to differentiate between plant genotypes and to estimate the amount of diversity and relatedness in a collection of genotypes. The introduction of isoenzymes was into herbal education in the early 1960s and evolved in importance in the 1970s and 1980s. In today's fingerprinting of plants, PCR-based multi-locus methods, PCR-based single-locus methods, single nucleotide polymorphisms, organellar DNA-based methods are carried out on process developments in the dense linkage for various plants, like the herbs are using all kinds of markers. In the already brief existence of molecular genetics and genomics, the introduction of massively parallel high throughput gene studies was a breathtaking phase forward 10 years ago.[2,8] On the other hand, DNA marker technologies are currently being successively complemented or even replaced by the sequencing process itself which is expressed as "genome sequencing".[2,10,11,12] The polymer chain reaction is shown in figure.2.and discussed in table.1.

Fig 2: PCR cycle and **Table 1.** PCR cycle

2.3 Today's DNA fingerprinting applications in Herbs are

- Identification of the genotype in wild plants, cultivars propagated vegetatively, and cultivars propagated with seed.
- Somatic mutation genotyping and in vitro propagated substance genotyping.
- Forensic Botanics
- Genetic diversity, population composition, gene flow, and differentiation of species and genetic interactions.
- Genome constitution; research into hybridization, introgression, and polyploidy.
- Phytogeography, plant speciation, systematics, and phylogeny.
- Genetic mapping-mapping of linkages, genetic maps, and mapping of associations.

Complex genotyping techniques (RAPD, AFLP, RFLP, and ISSR) are discussed in table.2.

Table 2: Complex genotyping techniques (RAPD, AFLP, RFLP, and ISSR).

2.4Techniques for Authentication of Herbal Plants Based on DNA

Herbal formulations are a type of medication comprising of one or more herbs or medicines that are designed in equal amounts to provide particular nutritional and cosmetic benefits for a cure, treatment, prevention, alteration of a physical or psychological structure or morphology. DNA-based methods have been widely used for the authentication of medicinal plant species. In cases where other individuals or varieties that are morphologically and/or phytochemicals distinguishable are often substituted or adulterated, this is particularly useful. DNA Techniques for authentication of herbal plants are explained in Table.3.

Table 3: DNA Techniques for authentication of herbal plants

Various DNA-based methods, including herbal remedies, involving the processing of raw herbs and/or herbal substances are discussed herein in table.4.

Table 4: Various DNA-based methods, including herbal remedies, involving the processing of raw herbs and/or herbal substances

2.5 DNA barcoding

DNA barcoding has been extensively used in many scientific fields, including ecology, evolutionary theory, cell biology, neuroscience, geochemistry, and bio-industry. The mitochondrial gene cytochrome c oxidase subunit1 (COI) was proposed as a barcode for species recognition in all rodents. Mitochondrial genes are mostly not appropriate for use as barcodes in plants once they have low nucleotide replication and lower evolutionary rates that are poorly involved in the identification of plant organisms. The universal barcode region should be introduced, expanded, and classifies all plant species, such as challenges for the several researchers are proposed genome regions with evolutionary rates than the mitochondrial genes such as barcodes for plants. DNA barcoding has shown substantial advances in medicinal plant research in recent years. 60 Besides, a broad variety of herbal products have been used as industrial quality assurance and authorization tools. Most of the herbs are used in the Japanese and Chinese pharmacopeia, the set of the procedure is applied, the authors proposed as a standard barcode for foreign applications and the safe use of herbs. DNA barcoding is used in raw botanicals and/or herbal product authentication; including herbal remedies are shown in table.5.

Table 5: DNA barcoding used in raw botanicals and/or herbal product authentication, including herbal remedies.

2.6 Real-time PCR and HRM analysis

The real-time PCR relies on the use of characteristic primers to amplify fragments of prey PCR, which is an important benefit for dietary herbal analysis that often accommodates highly degraded DNA. In several diagnostic and food analysis laboratories, it is the method of choice since it relies on real-time monitoring of product formation during reaction and accuracy by accurate probes or amplicon melting curve analysis. The combination of high sensitivity, accuracy, reliability, low cross-contamination, and reduced time analysis is the advantage of making real-time PCR an appealing alternative to traditional PCR. HRM (High-Resolution Melt) Analysis is a tool; It facilitates the gradual denaturation of PCR-amplified DNA since the temperature rises slowly and the genetic variants are analyzed. In real-time PCR applications, high-resolution instrumentation and new-generation fluorescent dyes are used. HRM is a method that is simple, cost-effective, high-performance, highly accurate, and sensitive. These developments were focused on alternative methods of Gene delivery, namely, sequence isothermal amplification (LAMP, HAD and NGS) New and enhanced sequencing technologies are categorized as faults of first-generation sequencing are discussed and techniques are explained in Figure.3.

Fig 3: Isothermal amplification methods

The application of real-time PCR and HRM for the identification of raw botanicals and/or herbal products, including herbal medicines, is discussed herein in Table.6.

Table 6: The application of real-time PCR and HRM for the identification of raw botanicals and/or herbal products, including herbal medicines

Advanced DNA-based techniques for the detection of raw herbs and/or herbal products, particularly natural supplements. (HDA, LAMP, NGS) are shown in table.7.

Table 7: For the authentication of natural herbs and/or herbal products, including herbal remedies, advanced DNA-based techniques (HDA, LAMP, and NGS).

3. CONCLUSION

DNA fingerprinting seems to have a wide variety of uses and is used for herbal security. It has been shown that DNA fingerprinting is a tool for the detection and identification of various adulterants and can distinguish between individuals, species, and populations. Fingerprinting techniques, PCR cycles, complex genotyping techniques, DNA-based authentication techniques for herbal plants

such as DNA techniques, DNA barcoding, real-time PCR & HRM analysis, and Advanced DNA-based strategies such as (HAD, LAMP, NGS) are used for the authorization of raw herbs and/or herbal products for the analysis of DNA interventions even if in a process, products were made from pills, powders, tablets or dried types. In this study, phytochemical consistency contains multiple given concerning the chemical composition present in herbal drugs. A Summary of DNA Finger Printing Techniques Used In the study of Herbal Products, DNA strategies fined things simple to standardize herbs to compare other techniques.

4. Outlook

The development of new bioactivity measures for concurrent engineering in natural plant science and industrial usage is a DNA-based authentication scheme for medicinal plants. This goal was accomplished through the advancement of DNA-based tools by providing a broad variety of tools, from easy and reliable approaches based on species-specific PCRs to advanced DNA barcoding and high-performance and multi-target Bioinformatics tools A critical feature of this software of DNA-based methods is the accuracy of DNA retrieved before the analysis. Large-scale identification of SNPs (single nucleotide polymorphisms) and SSRs (simple sequence repetitions) by greater sequencing approaches seems to have become standard, and highly addressable SNPs -based genotyping by decoding methods is already used for numerous technologies in many plant species and genetic elements. They can quickly realize that so many of the HMPs aren't powerful and that some can cause serious health problems. Awareness can also help increase consumer interest in the consumption of HMP, particularly if it is of a standard of excellence.

5. Abbreviations

DNA – Deoxyribose Nucleic Acid
RNA – Ribonucleic Acid
WHO – World Health Organisation
PCR –Polymerase Chain Reaction
LAMP – Loop-Mediated Isothermal Amplification
HDA – Helicase-Dependent Amplification
NGS – Next-Generation Sequencing
HRM – High-Resolution Melt Analysis
RFLP – Restriction Fragment Length Polymorphisms
AFLP – Amplified Fragment Length Polymorphisms
SCAR – Sequence Characterized Amplified Region
ISSR – Inter Simple Sequence Repeat
RAPD – Random Amplified Polymorphic DNA
SNPs – Single Nucleotide Polymorphisms
SSRs – Simple Sequence Repetitions
TLC – Thin Layer Chromatography
HPTLC – High-Performance Thin Layer Chromatography
COI - Cytochrome C Oxidase Subunit 1
RbcL - Ribulose 1, 5-Bisphosphate Carboxylase/Oxygenase Large Subunit
ITS -Internal Transcribed Spacer

ACKNOWLEDGMENT

The author would like to thanks RIPER management for the support of writing my review paper. Heart fully thanks to Dr.K. Vinodkumar Sir & S. Shakir Basha Sir for giving valuable suggestions to my review.

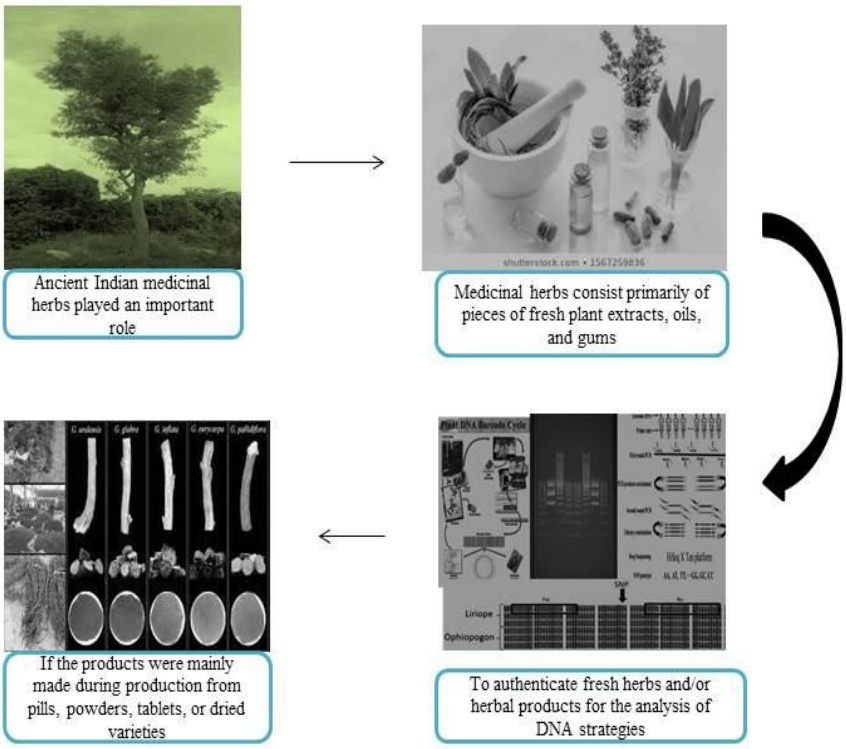

Table 1: PCR cycle

Polymerase Chain Reaction (PCR)		
1. Heat Denaturation	2. Annealing	3. Primer Extension
The double-stranded DNA in two distinct stands is expressed by all these levels. For 30 seconds, the degree of denatured proteins is 95 ° C or 97 ° C for 15 seconds, but warmer temperatures may also be appropriate for nucleic acids enriched in guanine and cytosine.	In that period, one primer binds to one DNA strand's 5 'end, as well as the other primer binds to its comparable strand's 3' end. Annealing is a single-stranded DNA hybridization of primers, or the length of time required for primers varies on the primers' target sequence, frequency, and quantity.	The temperature varies for Taq DNA polymerase, which one after another adds complement nucleic acids to the 3'OH primer group. At 72 °C, nucleotide integration predictions range from 100 nucleotides per second, depending on the pattern's pH level and DNA template consistency.
Denaturation at 94-96^{o}c (Cycle1)	Annealing at-68^{o}c (Cycle2)	Elongation at ca.72^{o}c (Cycle3)

Table 2: Complex genotyping techniques (RAPD, AFLP, RFLP, and ISSR)

The specific methods of genotyping (RAPD, AFLP, RFLP, ISSR) are applied and discussed	
Simple sequence repeats (SSRs)	Special replication primers (SSRs), 1 to 6 nucleotides in length, are microsatellites, indicating a high degree of polymorphism. SSRs can be marked by unique marking or by radio-labeling with electron microscopy, as with any DNA fragment.
Restriction fragment length polymorphisms (RFLP)	RFLPs are irregular fragment lengths of DNA obtained from cutting sequences of Fixed Amount of Tandem Repeat (VNTR) with restriction enzymes at specific sites up to 30 sequences long. Throughout this way, DNA magnification of PCR is not expected.
Amplified fragment length polymorphism (AFLP)	AFLP is also an RFLP-based method based on PCR that incorporates primers to enhance sequence selection. AFLP employs restriction enzymes for genomic DNA-cutting followed by an additive activation to the restriction fragments' sticky ends.
Random amplified polymorphic DNA (RAPD)	RAPD is among the major tests most frequently used to monitor for variations mostly in DNA sequences of several flowering plants. Through using random amplification, RAPD comprises fishing/searching the sequence. If another band of interest is calculated by a 2-dimensional run, the gel is cut and the DNA is retrieved and analyzed. If another band of interest is calculated by a 2-dimensional run, the gel is cut and the DNA is retrieved and analyzed.
Inter Simple Sequence Repeat (ISSR)	ISSR is a broad term for closely spaced microsatellites that are used as PCR primers with additional segments. Restricted amplification period length prevents unwanted replication during PCR of extremely long contiguous DNA sequences that can lead to a mixture of amplified DNA strands which are typically short but somewhat long.

Table 3: DNA Techniques for authentication of herbal plants

S.no	Title	Plant	Technique used	References
1	Authentication of the endangered medicinal content, Saussurea lappa, by ITS DNA and 5S rRNA sequencing	Saussurea lappa C.B Clarke.	Sequencing Based Markers	13
2	Genetic characterization and authentication by RAPD-PCR and SCAR marker of Embelia scales	Embelia ribes Burm.F.	SCAR	14
3	Development of SCAR marker for authentication of Pueraria tuberosa (Roxab. Ex. Willd.)DC	Pueraria tuberose Roxb.ex Wild.	SCAR	15
4	Cinnamon (Cinnamomum spp.) genetic identification based on trnL-trnF chloroplast DNA Genetic material	Cinnamomum zeylanicum	Sequencing	16
5	Aloe species recognition by spontaneous amplified polymorphic DNA (RAPD) analysis	Aloe arborescens Miller.	RAPD	17
6	Detection by real-time PCR of celery (Apium graveolens), mustard (Sinapis alba, Brassica juncea, Brassica nigra) and sesame (Sesamum indicum) in fruit.	Apium graveolens L.	Real time PCR	18
7	Application of SCAR (sequence characterized amplified region) analysis to authenticate *Lycium barbarum* (wolfberry) and its adulterants	Lycium barbarum L.	SCAR	19

Table 4: Various DNA-based methods, including herbal remedies, involving the processing of raw herbs and/or herbal substances

S.no	DNA Technique	Species of Plant	Material of the plant/ type of sample	References
1	Conventional PCR	Medicagosativa; Trifoliumpratense	Capsules	20
		Twenty-one Ephedraspp. (including E.sinica), Gnetumgnemon, Welwitschiamirabilis	Dried and fresh products, herbal mixtures, pills, capsules, aerial components,	21
		Echinaceaspp.; Matricaria chamomilla	Plant extracts, dried plant material	22
		Panaxginseng	Tablet, pills, or a liquid, and injection products	23
		Angelicasinensis, Panaxnotoginseng	Concentrated Chinese medicine granules	24
2	PCR-RFLP	Puerariacandolleivar.mirifica; Puerariacandolleivar.candollei; Buteasuperba; Mucunacollettii	Leaves, commercial herbal drugs	25
		Maytenusaquifolia; Maytenus ilicifolia	Leaves in natural and processed	26
		Terminaliachebulavar.chebula; T. chebulavar.nana; T.bellirica;T. catappaL.; T.citrina;Phyllanthus emblica; CombretumindicumL.	Leaves, fruits, powders	27
		Tetrastigmahemsleyanum	Fresh leaves	28
		Pulsatillachinensis	Leaves, roots, powder	29
3	ARMS	Panaxginseng	Leaves, single-taste medicines, compound medicinal preparation	30
		Cynanchumwilfordii; Polygonum multiflorum	Plant, seeds, herbal medicine	31
		Terminaliachebulavar.chebula;T. chebulavar.nana; T.bellirica; T. catappaL.; T.citrina; Phyllanthusemblica; CombretumindicumL	Leaves, fruits, powders	32
4	SCAR	Cynanchumwilfordii	Leaves, dry roots	33
		Aconitumheterophyllum	Dried tubers	34
		GardeniajasminoidesEllisvar. grandifloraNakai	Leaves, dried fruits	35
		Akebiaquinata; AkebiaTrifoliat	Fresh leaves, herbal medicines	36
		Araliacontinentalis; Angelica biserrata;	Leaves, herbal medicines	37
		Adenophorastricta; Adenophora triphylla	Leaves, herbal medicines	38

Table 5: DNA bar coding used in raw botanicals and/or herbal product authentication, including herbal remedies

s.no	Plant material/species	Plant material/ type of sample	Reference
1	5,905 genus of (medicinal plants and species closely associated with each other)	Leaves	38
2	85species belonging to Fabaceae family	Dried leaves and medicinal products for commercial use	39
3	(from 7 separate locations) Cinnamomum osmophloeum	Dried leaves	40
4	Rutagraveolens	Leaves	41
5	Panaxspecies, including but not limited to, P. bipinnatifidus, P.ginseng, P. japonicus, P.notoginseng, P. pseudoginseng, P.quinquefolius, P.stipuleanatus, and P.trifolius	Leaves	42
6	Boerhaviadiffusa	Leaves	43
7	Panaxspecies, including Other species, like Echinaceapurpúrea and Hypericumperforatum, are P. quinquefolius and P.ginseng.	Roots, tea, liquids, capsules, tablets	44
8	92species	Museum medicinal specimens	45
9	Lonicerajaponica / L.Macranthoides , L.L.hypoglauca; L.confuse; fulvotomentosa;	Dry flowers	45
10	Serenoarepens	Tissues from dried leaves and herbal supplements	46
11	44plantproductsrepresenting30 differentspecies 50leafsamplesfrom42plant species	Leaves, capsules, powder, tablet	47
12	2,431medicinalplantspecies	Dried leaves, dried stems	48
13	Gingkobiloba L.	Dry but rather granulated leaves, dried inorganic greenery harvest	49
14	20 species of Cassia, including C.tora, C.occidentalis, C.senna, and C.italic.	Leaves	50
15	Sennaalata; S.auriculata; S. siamea; S.atora; S. occidentalis; S.hirsuta;S. urattensis; S.polyphylla; S. uniflora; Cassiafistula; Chamaecristakleinii	Dried leaves, bark, fruits	51
16	Sidacordifolia	Branches, leaves, entire tree, stalks, seedlings etc.	52
17	131 samples of non-Aristolochiaceae (33 species of Clematis, Cocculus, Akebia, etc., including 20 genera from 12 families)	Dried material	53
18	1,436 raw herbal samples representing 295 medicinal species from 7 major TCM markets in China (including 515 radix etrhizoma samples, 451 fruit and seed samples, 115 herbal samples, 98 floss varieties, 82 stem samples, 93 cortex samples, 59 folium samples and 23 fungus samples)	Raw herb material	54
19	47 species	Roots, leaves, barks, powder	55
20	Fritillaria cirrhosa	Bulb's	56
21	Smithia conferta	Dried leaves	57
22	Eleuthero coccus senticosus; Rhodiolarosea	Roots, plant extracts	58
23	Garciniagummi-gutta (L.) Roxb., G.xanthochymus Hook.f.exT. Choisy, G.morella (Gaertn.) Desr. G.mangostanaL., G. Anderson, G.indica(Thouars)Buch.-Ham., G.lanceifolia Roxb., G.spicataHook.f., G.cowaRoxb.exChoisy., G.talbotiiRaizadaexSantapau, G.livingstoneiT.Anderson, G.pedunculataRoxb.ex.	Leaves (n=11 genus with 2 to 5 individuals for each organisms) and Garcinia gummi-gutta and/or G.indica herbal remedies (n=10, capsules and tablets) of just about any Indigofera e-commerce species.	59

Table 6: The application of real-time PCR and HRM for the identification of raw botanicals and/or herbal products, including herbal medicines

DNA technique	Species	Plant material/ type of sample	Reference
Real-time PCR (TaqManMGB probes)	Cynanchumwilfordii	Fresh leaves, dry roots	60
Real-time PCR(TaqManprobes)	(33 species Clematis, Cocculus, Akebia, etc., including 20 genera from 12 families) 131 specimens of non-Aristolochiaceae	Dried plant material	61
Real-time PCR (SYBR Green)	Ginseng from Panax (5 different cultivars) Ginseng from Panaxnoto.	Fresh leaves	62
Real-time PCR(TaqManprobes)	Juglansregia L., Matricariarecutita L., QuercusroburL.	Dried material from plants, finished medicinal products	63
HRM/Bar-HRM	Panax ginseng; P.quinquefolius	New leaves, fresh sources, popular products; (powder, pellets, extracts, dried roots, sugar or honey ginseng, beverages, tea powder)	64
	Phyllanthusamarus	Plant material, capsule, tablet, tea	65
	Acanthuse bracteatus; Andrographis paniculata; Rhinacanthus nasutus	New and mature plant material	66
	Thunbergia laurifolia	New and dried organic matter, professional collections	66
	Hypericumperforatum;Hypericum androsaemum	Leaves, seeds, herbal infusions	67
	Cynanchumwilfordii;Polygonum multiflorum	Plant, seeds, herbal medicine	68
	(Acanthaceae, Zingiberaceae, Leguminosae, Araliaceae, Vitaceae, Papilioneae, Cucurbitaceae, Moringaceae, Lamiaceae, Euphorbiaceaceae, Piperaceae, Compositae, Menispermaceae, Compositae) 96 popular Thai medicinal herbs	Plant material	66
	Artemisiaargyi, A.annua, A.indica, A.lavandulaefolia, A.atrovirens	Dry organic matter, agricultural herb commodities	69
	Sage (SalviaofficinalisL.), Greek sage (Salviafruticosa), chamomile (MatricariachamomillaL.), mountain-tea (Sideritisscardica), oregano (Origanumvulgare), Cretanoregano (Oreganoonites), yarrow (Achilleamillefolium), lemonbalm (Melissaofficinalis) and rosemary (Rosmarinus officinalis)	Plant tissue	70
	Dendrobium officinale; Dendrobium huoshanense;	Stem, leaves (decoction pieces and Dendrobium material) and commercial samples	71
	Glycyrrhiza uralensis; glabra glycyrrhiza; inflata Glycyrrhiza	Leaves, commercial herbal products	72
	Senna alexandrina	Plant tissue and commercial samples	73
	Tinosporacrispa	Dried plant tissue, tablets, capsules	74
	Annonamuricata (soursop)	Leaves, commercial herbals products	74

Table 7: For the authentication of natural herbs and/or herbal products, including herbal remedies, advanced DNA-based techniques (HDA, LAMP, and NGS)

DNA technique	Species	Plant material/ type of sample	Reference
HAD	Panax ginseng;P. quinquefolius; P.noto ginseng	Powder, dried roots, tea granules, regular materials	75
LAMP	Curcumalonga; Curcuma aromatic	rhizomes	76
	Panax ginseng; Glycyrrhiza uralensis; Panax japonicas	Plant material, herbal medicines	77
	Nigellasativa; Catharanthus roseus	Seeds	78
	Hedyotisdifusa	Herbal materials	79
	Taraxacumformosanum;	Dried leaves	80
	Crocus sativus L.	Leaves, roots, stigmas, filaments, petals	81
NGS/ metabarcoding	From 68 families, several different species	Flakes, powder, pills, tablets, herbal tea	82
	Rehmanniaglutinosa, Cornus officinalis, Dioscorea opposita, Poriacocos; Paeonia suffruticosa; Alismaorientalis	Traditional Chinese medicines	83
	Echinacea purpurea, Valeriana officinalis, Ginkgobiloba, Hypericumperforatum, Trigonellafoenum-graecum	Dry aerial materials, seeds and roots; extracts of plant, samples combined with raw resources of plants, content with or without supplements;	84
	17 (Angiospermae and Cycadopsida) plant species	Plant components, dynamic mixtures of models, industrial specimens of traditional medicinal products (capsules)	85
	Veronica officinalis L	Dried leaves, herbalteas, extracts, capsules, candies	86
	H.perforatum	Herbal teas, capsules, tablets, extracts	87

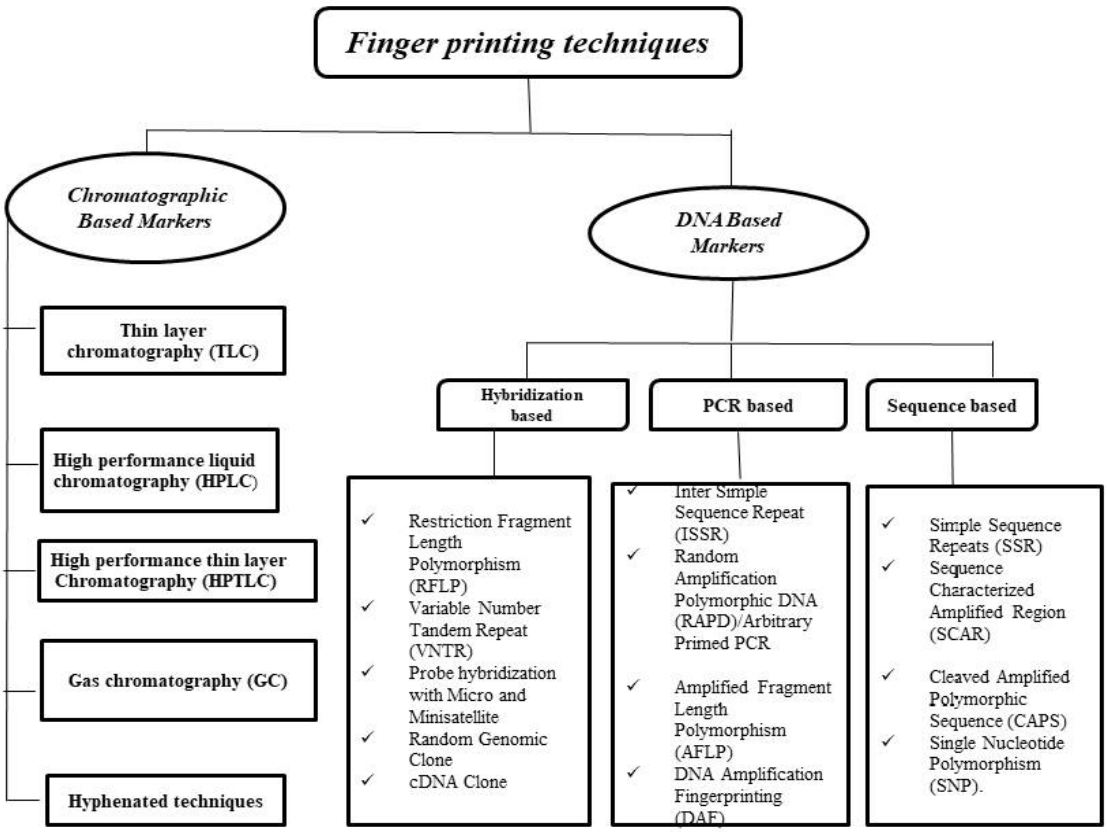

Fig. 1: Finger printing techniques

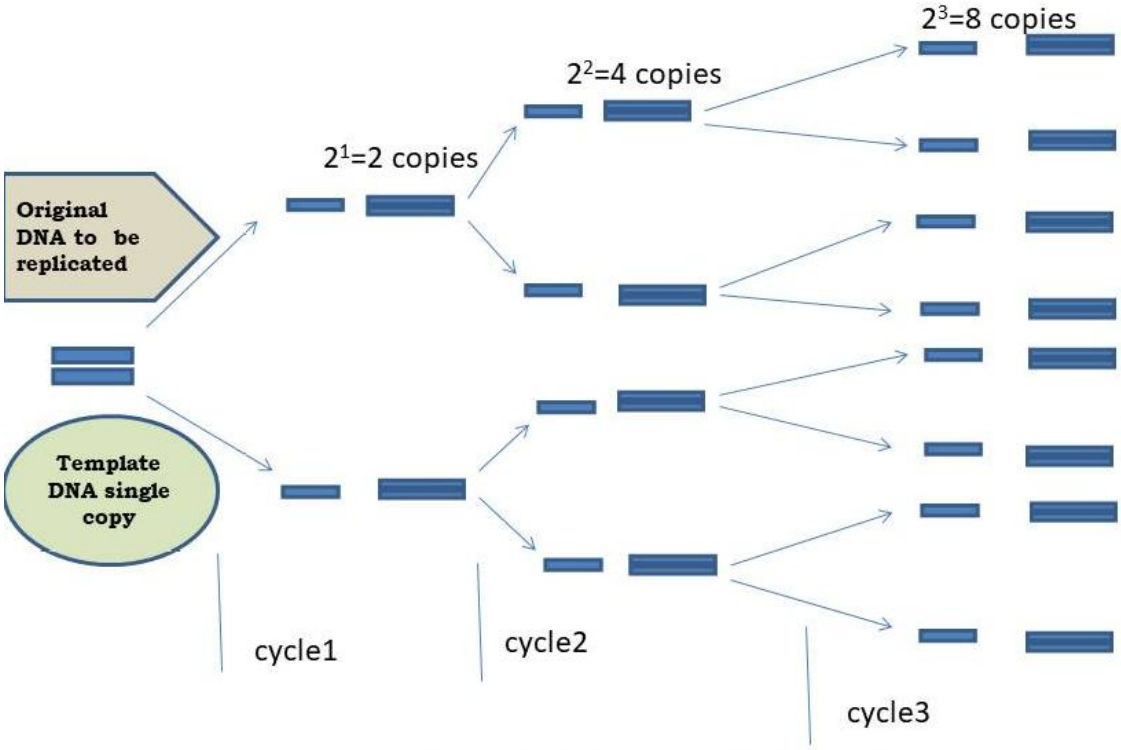

Fig. 2: Polymerase chain reaction

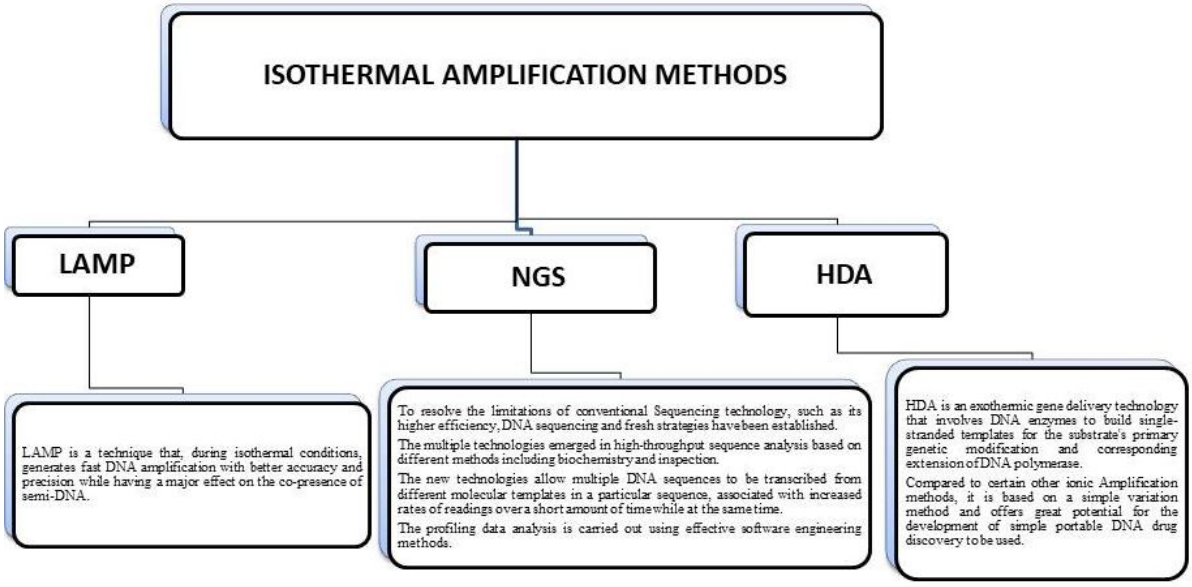

Fig. 3: Isothermal amplification methods

6. REFERENCES

1. Kumar PS, Ketkar P, Nayak S and Roy S. Application of DNA fingerprinting tools for authentication of ayurvedic herbal medicines–A review. J Sci Innov Res. 2014;3:606-12.
2. Nybom H, Weising K and Rotter B. DNA fingerprinting in botany: past, present, future. Investigative genetics. 2014;5 (1):1.
3. Grazina L, Amaral JS and Mafra I. Botanical origin authentication of dietary supplements by DNA based approaches. Comprehensive Reviews in Food Science and Food Safety. 2020;19(3):1080-109.
4. Indhu Rekka NC, Sathiyawathie RS and Gurunathan D. DNA probe. Drug Invention Today. 2019;12 (3).
5. Sharma A, Namdeo A and Mahadik K. Molecular markers: New prospects in plant genome analysis. Pharmacognosy reviews. 2008;2(3):23.
6. Quality control methods for medicinal plant material, World health organization guidelines. 1998.
7. DNA fingerprinting in plants, http://biosolutions.6te.net/articles_files/dna_fin.html.
8. Sambrook J, Fritsch EF and Maniatis T. Molecular cloning: a laboratory manual, cold spring harbor laboratory, cold spring harbor laboratory press, 2nd ed. New York. 1989;1659.
9. Harish Vasudevan. DNA fingerprinting in the standardization of herbs and nutraceuticals, the science creative quarterly. 2011;6. http://www.scq.ubc.ca/dna-fingerprinting-in-the-standardization-ofherbs-and nutraceuticals.
10. Mardis ER. Next-generation DNA sequencing methods. Annu Rev Genomics Hum Genet 2008.
11. Metzker ML. Sequencing technologies- the next generation. Nature Rev Genet. 2010.
12. Rothberg JM and Leamon JH. The development and impact of 454 sequencings. Nature Biotechnol. 2008.
13. Chen F, Chan HYE, Wong KL, Wang J, Yu MT, But PPH and Shaw PC. Authentication of Saussurea lappa, an endangered medicinal material, by ITS DNA and 5S rRNA sequencing. Planta Medica. 2008;74:889-892.
14. Devaiah KM and Venkatasubramanian P. Genetic characterization and authentication of Embelia Ribes using RAPD-PCR and SCAR marker. Planta Medica. 2008;7:194-196.
15. Devaiah KM and Venkatasubramanian P. Development of SCAR marker for authentication of Pueraria tuberose (Roxb. ex. Willd.) DC. Current Science. 2008;94:1306-1309.
16. Kojoma M, Kurihara K, Yamada K, Sekita S, Satake M and Iida O. Genetic identification of cinnamon (Cinnamomum spp.) based on the trnL-trnF chloroplast DNA. Planta Medica. 2002;68:94-96.
17. Shioda H, Satoh K, Nagai F, Okubo T, Seto T, Hamano T, Kamimura H and Kano I. Identification of Aloe species by random amplified polymorphic DNA (RAPD) analysis. Journal of the Food Hygienic Society of Japan. 2003;44:203-207.
18. Mustorp S, Axelsson CE, Svensson U and Holck A. Detection of celery(Apium graveolens), mustard (Sinapis alba, Brassica juncea, Brassica nigra) and sesame (Sesamum indicum) in food by real-time PCR. European Food Research and Technology. 2008;226:771-778.
19. Sze SC, Song JX, Wong RN, Feng YB, Ng TB, Tong Y and Zhang KY. Application of SCAR (sequence characterized amplified region) analysis to authenticate Lycium barbarum (wolfberry) and its adulterants. Biotechnology and Applied Biochemistry. 2008;51:15.
20. LeRoy A, Potter E, Woo HH, Heber D and Hirsch AM. Characterization and identification of Alfalfa and red clover dietary supplements using a PCR-based method. Journal of Agricultural and Food Chemistry. 2002;50(18):5063–5069. https://doi.org/10.1021/jf0255634.
21. Techen N, Khan IA, Pan Z and Scheffler BE. The use of polymerase chain reaction (PCR) for the identification of Ephedra DNA in dietary supplements. Planta Medica. 2006;72(03):241–247. https://doi.org/10.1055/s-2005-916173.
22. Novak J. Grausgruber-Gröger S and Lukas B. DNA-based authentication of plant extracts. Food Research International. 2007;40(3):388–392. https://doi.org/10.1016/j.foodres.2006.10.015.

23. Chen R, Dong J, Cui X, Wang W, Yasmeen A, Deng Y and Tang Z. DNA-based identification of medicinal materials in Chinese patent medicines. Scientific Reports. 2012;2:958. https://doi.org/10.1038/srep00958
24. Lo YT and Shaw PC. DNA barcoding in concentrated Chinese medicine granules using adaptor ligation-mediated polymerase chain reaction. Journal of Pharmaceutical and Biomedical Analysis. 2018b;149:512–516.
25. Wiriyakarun S, Yodpetch W, Komatsu K, Zhu S, Ruangrungsi N and Sukrong S. Discrimination of the Thai rejuvenating herbs Pueraria candollei (White Kwao Khruea), Butea superba (Red Kwao Khruea), and Mucuna collettii (Black Kwao Khruea) using PCR-RFLP. Journal of Natural Medicines. 2013;67(3):562–570. https://doi.org/10.1007/s11418-012-0716-1
26. Nakamura SS, do Valle JS, Jacomassi E, Linde GA and Colauto NB. Molecular authentication of Maytenus sp by PCR-RFLP. Semina-Ciencias Agrarias. 2013;34(2):627–633. https://doi.org/10.5433/1679-0359.2013v34n2p627.
27. Peng X, Wu X, Ji Q, Yang R and Li Y. Molecular authentication of Tetrastigmahemsleyanum from its adulterant species using ISSR, CAPS, and ITS2 barcode. Molecular Biology Reports. 2016;43(8):785–794. https://doi.org/10.1007/s11033-016-4023-x
28. Shi Y, Zhao M, Yao H, Yang P, Xin T, Li B and Chen S. Rapidly discriminate commercial medicinal Pulsatilla chinensis (Bge.) Regel from its adulterants using ITS2 barcoding and specific PCR-RFLP assay. Scientific Reports. 2017;7:40000. https://doi.org/10.1038/srep40000.
29. Diao Y, Lin XM, Liao CL, Tang CZ, Chen ZJ and Hu ZL. Authentication of Panax ginseng from its adulterants by PCR-RFLP and ARMS. Planta Medica. 2009;75(5):557–560. https://doi.org/10.1055/s-0029-1185321.
30. Han EH, Cho K, Goo Y, Kim M, Shin YW, Kim YH and Lee SW. Development of molecular markers, based on chloroplast and ribosomal DNA regions, to discriminate three popular medicinal plant species, Cynanchumwilfordii, Cynanchumauriculatum, and Polygonum multiflorum. Molecular Biology Reports. (2016a);43(4):323–332. https://doi.org/10.1007/s11033-016-3959-1.
31. Intharuksa A, Ando H, Miyake K, Sirisa-Ard P, Mikage M and Sasaki Y. Molecular analysis of Terminalia spp. distributed in Thailand and authentication of crude drugs from Terminalia plants. Biological and Pharmaceutical Bulletin. 2016;39(4):492–501. https://doi.org/10.1248/bpb.b15-00673.
32. Ryuk JA, Lee HW, Ju YS and Ko BS. Monitoring and identification of Cynanchum wilfordii and Cynanchum auricuLatum by using molecular markers and real-time polymerase chain reaction. Journal of the Korean Society for Applied Biological Chemistry. 2014;57(2):245–251. https://doi.org/10.1007/s13765-013-4248-5
33. Seethapathy GS, Balasubramani SP and Venkatasubramanian P. nrDNA ITS sequence-based SCAR marker to authenticate Aconitum heterophyllum and Cyperus rotundus in Ayurvedic raw drug source and prepared herbal products. Food Chemistry. 2014;145:1015–1020. https://doi.org/10.1016/j.foodchem.2013.09.027
34. Mei Z, Zhou B, Wei C, Cheng J, Imani S, Chen H and Fu J. Genetic authentication of Gardeniajasminoides Ellisvar. grandiflora Nakai by improved RAPD-derived DNA markers. Molecules. 2015;20(11):20219–20229. https://doi.org/10.3390/molecules 201119687.
35. Moon BC Ji, Lee Y, Kang YM and Kim HK. Authentication of Akebia quinata DECNE. From its common adulterant medicinal plant species based on the RAPD-derived SCAR markers and multiplex-PCR. Genes & Genomics. 2015;37(1):23–32. https://doi.org/10.1007/s13258-014-0225-6.
36. Kim WJ, Moon BC, Yang S, Han KS, Choi G and Lee AY. Rapid authentication of the herbal medicine plant species AraliacontinentalisKitag. And AngelicabiserrataC.Q. Yuan and R.H. Shan using ITS2 sequences and multiplex-SCAR markers. Molecules.

2016;21(3):270. https://doi.org/10.3390/molecules21030270.
37. Moon BC, Kim W, Han K, Yang S, Kang Y, Park I and Piao R. Differentiating authentic adenophorae radix from its adulterants in commercially-processed samples using multiplexed ITS sequence-based SCAR markers. Applied Sciences. 2017; 7(7):660. https://doi.org/10.3390/app7070660
38. Chen S, Yao H, Han J, Liu C, Song J, Shi L and Leon C. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS ONE. 2010;5(1):e8613. https://doi.org/10.1371/journal.pone.0008613.
39. Gao T, Yao H, Song J, Liu C, Zhu Y, Ma X and Chen S. Identification of medicinal plants in the family Fabaceae using a potential DNA barcode ITS2. Journal of Ethnopharmacology. 2010;130(1):116–121. https://doi.org/10.1016/j.jep.2010.04.026.
40. Lee SC, Chiou SJ, Yen JH, Lin TY, Hsieh KT and Yang JC. DNA barcoding Cinnamomum osmophloeum Kaneh. based on the partial non-coding ITS2 region of ribosomal genes. Journal of Food and Drug Analysis. 2010;18(2):128–135.
41. Al-Qurainy F, Khan S, Tarroum M, Al-Hemaid FM and Ali MA. Molecular authentication of the medicinal herb Ruta graveolens (Rutaceae) and an adulterant using nuclear and chloroplast DNA markers. Genetics and Molecular Research. 2011;10(4):2806–2816. https://doi.org/10.4238/2011.November.10.3
42. Zuo Y, Chen Z, Kondo K, Funamoto T, Wen J and Zhou S. DNA barcoding of Panax species. Planta Medica. 2011;77(2):182–187. https://doi.org/10.1055/s-0030-1250166.
43. Selvaraj D, Shanmughanandhan D, Sarma RK, Joseph JC, Srinivasan RV and Ramalingam S. DNABarcodeITSeffectively distinguishes the medicinal plant Boerhavia diffusa from its adulterants. Genomics, Proteomics & Bioinformatics. 2012;10(6):364–367. https://doi.org/10.1016/j.gpb.2012.03.002.
44. Wallace LJ, Boilard SMAL, Eagle SHC, Spall JL, Shokralla S and Hajibabaei M. DNA barcodes for everyday life: Routine authentication of natural health products. Food Research International. 2012;49(1):446–452. https://doi.org/10.1016/j.foodres.2012.07.048.
45. Han J, Zhu Y, Chen X, Liao B, Yao H, Song J and Meng F. The short ITS2 sequence serves as an efficient taxonomic sequence tag in comparison with the full-length ITS. BioMed Research International. 2013. https://doi.org/10.1155/2013/741476.
46. Little DP and Jeanson ML. DNA barcode authentication of saw palmetto herbal dietary supplements. Scientific Reports. 2013;3518. https://doi.org/10.1038/srep03518.
47. Newmaster SG, Grguric M, Shanmughanandhan D, Ramalingam S and Ragupathy S. DNA barcoding detects contamination and substitution in North American herbal products. BMC Medicine. 2013;1(1):222. https://doi.org/10.1186/1741-7015-11-222.
48. Pang X, Shi L, Song J, Chen X and Chen S. Use of the potential DNA barcode ITS2 to identify herbal materials. Journal of Natural Medicines. 2013;67(3):571–575. https://doi.org/ 10.1007/s11418-012-0715-2.
49. Little DP. Authentication of Ginkgo biloba herbal dietary supplements using DNA barcoding. Genome. 2014b:57(9):513–516. https://doi.org/10.1139/gen-2014-0130.
50. Purushothaman N, Newmaster SG, Ragupathy S, Stalin N, Suresh D, Arunraj DR and Parani M. A tiered barcode authentication tool to differentiate medicinal Cassia species in India. Genetics and Molecular Research. 2014;13(2):2959–2968. https://doi.org/10.4238/2014.April.16.4.
51. Seethapathy GS, Ganesh D, Kumar Santhosh UJ, Senthilkumar U, Uma Shaanker R and Ravikanth G. Assessing product adulteration in natural health products for laxative yielding plants, Cassia, Senna, and Chamaecrista, in Southern India using

DNA barcoding. International Journal of Legal Medicine. 2015;129(4):693–700. https://doi.org/10.1007/s00414-014-1120-z.
52. Vassou SL, Kusuma G and Parani M. DNA barcoding for species identification from dried and powdered plant parts: A case study with authentication of the raw drug market samples of Sida cordifolia. Gene. 2015;559(1):86–93. https://doi.org/10.1016/j.gene.2015.01.025.
53. Wu L, Sun W, Wang B, Zhao H, Li Y, Cai S and Chen S. An integrated system for identifying the hidden assassins in traditional medicines containing aristolochic acids. Scientific Reports. 2015;5. https://doi.org/10.1038/srep11318.
54. Han EH, Cho K, Goo Y, Kim M, Shin YW, Kim YH and Lee SW. Development of molecular markers, based on chloroplast and ribosomal DNA regions, to discriminate three popular medicinal plant species, Cynanchumwilfordii, Cynanchumauriculatum, and polygonum multiflorum. Molecular Biology Reports. 2016a;43(4):323–332. https://doi.org/10.1007/s11033-016-3959-1.
55. Michel CI, Meyer RS, Taveras Y and Molina J. The nuclear internal transcribed spacer (ITS2) as a practical plant DNA barcode for herbal medicines. Journal of Applied Research on Medicinal and Aromatic Plants. 2016;3(3):94–100. https://doi.org/10.1016/j.jarmap.2016.02.002.
56. Xiang L, Su Y, Li X, Xue G, Wang Q, Shi J and Chen S. Identification of Fritillariae bulbs from adulterants using ITS2 regions. Plant Gene. 2016;7:42–49. https://doi.org/10.1016/j.plgene.2016.05.001.
57. Umdale SD, Kshirsagar PR, Lekhak MM and Gaikwad NB. Molecular authentication of the traditional medicinal plant “LakshmanBooti” (SmithiaconfertaSm.)and its adulterants through DNA barcoding. Pharmacognosy Magazine. 2017;13(50):S224–S229. https://doi.org/10.4103/pm.pm_499_16.
58. Ruhsam M and Hollingsworth PM. Authentication of Eleutherococcus and Rhodiola herbal supplement products in the United Kingdom.Journal of Pharmaceutical and Biomedical Analysis. 2018;149:403–409. https://doi.org/10.1016/j.jpba.2017.11.025.
59. Seethapathy GS, Tadesse M, Urumarudappa SKJ, Gunaga SV, Vasudeva R, Malterud KE and Wangensteen H. Authentication of Garcinia fruits and food supplements using DNA barcoding and NMR spectroscopy. Scientific Reports. 2018; 8. https://doi.org/10.1038/s41598-018-28635-z.
60. Ryuk JA, Lee HW, Ju YS and Ko BS. Monitoring and identification of Cynanchum wilfordii and Cynanchum auricuLatum by using molecular markers and real-time polymerase chain reaction. Journal of the Korean Society for Applied Biological Chemistry. 2014;57(2):245–251. https://doi.org/10.1007/s13765-013-4248-5.
61. Wu L, Sun W, Wang B, Zhao H, Li Y, Cai S and Chen S. An integrated system for identifying the hidden assassins in traditional medicines containing aristolochic acids. Scientific Reports. 2015;5. https://doi.org/10.1038/srep11318.
62. Wang H, Wang J and Li G. A simple real-time polymerase chain reaction (PCR)-based assay for authentication of the Chinese Panax ginseng cultivar Damaya from a local ginseng population. Genetics and Molecular Research. 2016;15(2). https://doi.org/10. 4238/gmr.15028801
63. Doganay-Knapp K, Orland A, König GM Knöss W. The potential of three different PCR-related approaches for the authentication of mixtures of herbal substances and finished herbal medicinal products. Phytomedicine. 2018;43:60–67. https://doi.org/10.1016/j.phymed.2018.03.062.
64. Jung J, Kim KH, Yang K, Bang KH and Yang TJ. Practical application of DNA markers for high-throughput authentication of Panax ginseng and Panax quinquefolius from commercial ginseng products. Journal of Ginseng Research. 2014;38(2):123–129. https://doi.org/10.1016/j.jgr.2013.11.017.
65. Buddhachat K, Osathanunkul M, Madesis P, Chomdej S and Ongchai S. Authenticity analyses of

Phyllanthus amarus using barcoding coupled with HRM analysis to control its quality for medicinal plant products. Gene. 2015;573(1):84–90. https://doi.org/10.1016/j.gene.2015.07.046.

66. Osathanunkul M, Suwannapoom C, Osathanunkul K, Madesis P and de Boer H. Evaluation of DNA barcoding coupled high resolution melting for discrimination of closely related species in phytopharmaceuticals. Phytomedicine. 2016;23(2):156–165. https://doi.org/10.1016/j.phymed.2015.11.018.
67. Costa J, Campos B, Amaral JS, Nunes ME, Oliveira MB and Mafra I. HRM analysis targeting ITS1 and matK loci as potential DNA mini-barcodes for the authentication of Hypericum perforatum and Hypericum androsaemum in herbal infusions. Food Control. 2016;1;61:105-14.
68. Han EH, Cho K, Goo Y, Kim M, Shin YW, Kim YH and Lee SW. Development of molecular markers, based on chloroplast and ribosomal DNA regions, to discriminate three popular medicinal plant species, Cynanchumwilfordii, Cynanchumauriculatum, and Polygonum multiflorum. Molecular Biology Reports. 2016a;43(4):323–332. https://doi.org/10.1007/s11033-016-3959-1.
69. Song M, Li J, Xiong C, Liu H and Liang J. Applying high-resolution melting (HRM) technology to identify five commonly used Artemisia species. Scientific Reports. 2016;6:34133. https://doi.org/10.1038/srep34133
70. Xanthopoulou A, Ganopoulos I, Kalivas A, Osathanunkul M, Chatzopoulou P, Tsaftaris A and Madesis P. Multiplex HRM analysis as a tool for rapid molecular authentication of nine herbal teas. Food Control. 2016;60:113–116. https://doi.org/10.1016/j.foodcont.2015.07.021
71. Dong X, Jiang C, Yuan Y, Peng D, Luo Y, Zhao Y and Huang L. Application of high-resolution melting analysis for authenticity testing of valuable Dendrobium commercial products. Journal of the Science of Food and Agriculture. 2018;98(2):549–558. https://doi.org/10.1002/jsfa.8496.
72. Li J, Xiong C, He X, Lu Z, Zhang X, Chen X and Sun W. Using SSR-HRM to identify closely related species in herbal medicine products: A case study on licorice. Frontiers in Pharmacology. 2018;9:407. https://doi.org/10.3389/fphar.2018.00407.
73. Mishra P, Shukla AK and Sundaresan V. Candidate DNA barcode tags combined with high resolution melting (Bar-HRM) curve analysis for authentication of Senna alexandrina Mill. with validation in crude drugs. Frontiers in Plant Science. 2018; 9:283. https://doi.org/10.3389/fpls.2018.00283.
74. Osathanunkul M, Osathanunkul R and Madesis P. Species identification approach for both raw materials and end products of herbal supplements from Tinospora species. BMCComplementary and Alternative Medicine. 2018;18(1):111. https://doi.org/ 10.1186/s12906-018-2174-0.
75. Kim YJ, Zhang D and Yang DC. Biosynthesis and biotechnological production of ginsenosides. Biotechnology advances. 2015;33(6):717-35.
76. Sasaki Y and Nagumo S. Rapid Identification of Curcuma longa and C. aromatica by LAMP. Biological and Pharmaceutical Bulletin. 2007;30(11):2229–2230. https://doi.org/10.1248/bpb.30. 2229
77. Sasaki Y, Komatsu K and Nagumo S. Rapid detection of Panax ginseng by loop-mediated isothermal amplification and its application to authentication of ginseng. Biological and Pharmaceutical Bulletin. 2008;31(9):1806–1808. https://doi.org/10.1248/bpb.31. 1806.
78. Ganie SH, Yadav D, Ahmad A, Chadhry A and Asif A. Authentication of traditional crop Kalongi (Nigella sativa L.) by LAMP marker. Indian Journal of Research in Pharmacy and Biotechnology. 2013;1:765–771.
79. Li M, Wong YL, Jiang LL, Wong KL, Wong YT, Lau CBS and Shaw PC. Application of novel loop-mediated isothermal amplification (LAMP) for rapid authentication of the herbal tea ingredient HedyotisdiffusaWilld. FoodChemistry. 2013;141(3): 2522–2525.

https://doi.org/10.1016/j.foodchem.2013.05.085.
80. Lai GH, Chao J, Lin, MK, Chang WT, Peng WH, Sun FC and Lee MS. Rapid and sensitive identification of the herbal tea ingredient Taraxacum formosanum using loop-mediated isothermal amplification. International Journal of Molecular Sciences. 2015;16(1):1562–1575. https://doi.org/10.3390/ijms16011562
81. Zhao M, Shi Y, Wu L, Guo L, Liu W, Xiong C and Chen S. Rapid authentication of the precious herb saffron by loop-mediated isothermal amplification (LAMP) based on internal transcribed spacer 2 (ITS2) sequence. Scientific Reports. 2016;6. https://doi.org/10.1038/srep25370.
82. Coghlan ML, Haile J, Houston J, Murray DC, White NE, Moolhuijzen P and Bunce M. Deep sequencing of plant and animal DNA contained within traditional Chinese medicines reveals legality issues and health safety concerns. PLoS Genetics. 2012;8(4):e1002657. https://doi.org/10.1371/journal.pgen.1002657
83. Cheng X, Su X, Chen X, Zhao H, Bo C, Xu J and Ning K. Biological ingredient analysis of traditional Chinese medicine preparation based on high-throughput sequencing: The story for Liuwei Dihuang Wan. Scientific Reports. 2014;4:5147. https://doi.org/10.1038/srep05147.
84. Ivanova NV, Kuzmina ML, Braukmann TWA, Borisenko AV and Zakharov EV. Authentication of herbal supplements using next-generation sequencing. PLoS ONE. 2016;11(5):e0156426. https://doi.org/10.1371/journal.pone.0156426.
85. Arulandhu AJ, Staats M, Hagelaar R, Voorhuijzen MM, Prins TW, Scholtens I and Kok E. Development and validation of a multi-locus DNA metabarcoding method to identify endangered species in complex samples. Giga Science. 2017;6(10):1–18. https://doi.org/10.1093/gigascience/gix080
86. Raclariu AC, Mocan A, Popa MO, Vlase L, Ichim MC, Crisan G and de Boer H. Veronica officinalis product authentication using DNA metabarcoding and HPLC-MS reveals widespread adulteration with Veronicachamaedrys. Frontiers in Pharmacology. 2017a;8:378. https://doi.org/10.3389/fphar.2017.00378.
87. Raclariu AC, Paltinean R, Vlase L, Labarre A, Manzanilla V, Ichim MC and de Boer H. Comparative authentication of Hypericum perforatum herbal products using DNA meta barcoding, TLC, and HPLC-MS. Scientific Reports. 2017b;7(1):1291. https://doi.org/10.1038/s41598-017-01389-w.