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Application of Next Generation Sequencing Technologies for Authentication of Herbal Products: A Comprehensive Review

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Next Generation Sequencing, therapeutic plants, authentication, metabarcoding.

ABSTRACT:

Molecular authentication of medicinal plants is essential to ensure product quality, safety, and efficacy. Next Generation Sequencing (NGS) has emerged as a powerful tool for accurate and high-throughput species identification, offering advantages over traditional methods. By analysing the DNA sequences of medicinal plants, NGS enables researchers to accurately identify and verify plant species, ensuring the quality and effectiveness of herbal remedies. The accurate recognition of taxa in heterogeneous samples is additionally possible by metabarcoding technique, which has been developed through next-generation sequencing. This review delves into the various NGS techniques along with metabarcoding technique for molecular authentication of medicinal plants, including principle, methodologies, challenges, and future prospects of NGS in the authentication of medicinal plants, emphasizing the importance of this technology in preserving the integrity of herbal medicines.

1. Introduction

Over thousands of decades, therapeutic plants along with herbal products have been crucial to maintaining the well-being of mankind. The usage and commercialization of therapeutic herbs are now heavily depends on authenticity and validation of accurate plant-based products [1]. Before making drugs, it is essential to confirm the veracity of the natural components implemented as medicines [2]. Because of illegal and irresponsible natural harvesting methods as well as the degradation of their natural environment, the population of therapeutic herbs is currently declining at a rapid pace [3]. Previous studies have shown that misidentification, adulteration, and substitution of medicinal plants are common problems in the industry, leading to ineffective treatments and potential health risks for consumers. The safety of the emerging herbal products and their authenticity are two of the current pharmacognosy's primary concerns [4]. The recognition of therapeutic herbs by molecular techniques that rely on the differences in DNA sequences have been considered as milestone in identification of species throughout the beginning of the 1990s. Molecular techniques have been established to differentiate real plants from contaminants [5, 6, 7]. An increasing number of research on the recognition of multiherb products have been reported as a result of advances in molecular methods and next-generation sequencing [8]. In order to address the drawbacks of Sanger sequencing for DNA barcoding of processed samples, next-generation sequencing (NGS) technique has been employed [9].

The advent of next-generation sequencing techniques decreased worker expenses by removing the necessity for cloning and subcloning along with producing huge quantities of information with simultaneous processing [10]. The significance of medicinal plants is revealed by NGS technology, a developing discipline that has been around for ten years. Additionally, it opens the door for the cultivation of therapeutic plants by molecular breeding, micropropagation, large-scale tissue culture as well as additional techniques for protecting medicinally significant plants [11]. With relatively little expense and efforts, NGS technology is providing a platform for studying the genomic, transcriptomic, and epigenomic components of non-model or therapeutic plants [12]. The identification of significant characteristics and genes involved in diverse biochemical processes of

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therapeutic plants has been made possible mainly due to the application of next generation sequencing [11]. NGS has made it possible to use the entire chloroplast genome sequence as the plant's super-barcode to identify closely related plants [13, 14].

It wasn't viable to use NGS as an industrial platform till 2005. Solexa Technologies introduced the initial platform in this category. Subsequently, an array of sequencing technologies had been created, exhibiting notable advancements. The most popular NGS technologies include Roche's 454 Pyrosequencing, Illumina Sequencing technology and Thermo Fisher Scientific's Ion Torrent Sequencing technology [15, 16]. Additionally growing in popularity, Oxford Nanopore Technology's (ONT), Nanopore Sequencing is actively utilised for assembling genomes with ultralong reads as well as for amplicon sequencing [17, 18]. The application of intricate algorithms for screening, assembling along with sequence assessment are crucial to NGS technology. The information gathered by analysis of NGS data is currently possible with the majority of readily accessible devices including DNA Star, CLC Bio, and Gene Spring [19]. An increasing demand has been seen in sequencing transcriptomes and genomes of potentially important herbs as NGS technology develop. A highly sophisticated and potent method for exploring transcripts in both model and non-model species is RNA sequencing or RNA-Seq [20]. Due to their short read lengths, Illumina and Ion Torrent Sequencing continue to be the most popular NGS methods for improving medicinal plants [11]. In a single sequencing run, it can produce up to one million DNA sequences with a maximum length of 700 bases, however the base length varies greatly according to the type of NGS platform being used.

Several species' amplicons and the identification of various species in a single sample was initially used by Coghlan et al. to identify Chinese medicine items. Using high-throughput sequencing and amplification of the *trnL* c/h region, they were able to identify 68 plant genera among 13 multi-ingredient samples. Since then, platforms of the second (primarily Illumina and Ion Torrent) and third generations (PacBio and Oxford Nanopore Technology) NGS technologies have been utilised for sequencing and for the generation of a reference genome assembly or the verification of different herbal supplements [8]. The original purpose of the NGS platforms was to produce DNA sequencing data from complete genomes or environmental samples. For instance, Parks et al. reconstructed the entire plastomes of 37 Pinus species on a multiplex Illumina sequencing platform and Moore et al. acquired the entire chloroplast sequence of *Ceratophyllum demersum* using the 454 Pyrosequencing technology. Through parallel sequencing the barcodes of several medicinal products, the NGS approach also proved helpful in confirming the contents of those products [21]. The techniques used for NGS have come a long way, from pyrosequencing to nanopore sequencing, and they are still constantly developing and expanding. The NGS market is becoming more competitive, which will lead to more affordable, quicker, and reliable data [10]. NGS-based metabarcoding is useful as well as growing in demand for determining the type of alteration caused by different plant-based goods [22, 23]. This review emphasizes the different next generation sequencing technologies along with metabarcoding technique for molecular authentication of therapeutic plants.

2. Principle Of Next Generation Sequencing Technology

In genomics studies, next-generation sequencing (NGS) is a robust technique. Millions of DNA fragments are capable of being sequenced simultaneously by NGS, yielding comprehensive data on genome organisation, genetic variants, gene activity, and modifications to genes [24]. The fundamental concept behind the method of NGS is the simultaneous sequencing of millions of short DNA fragments [11]. Several bioinformatics techniques are applied to the output analysis. After that, each individual read is mapped and organised in accordance with the accessible reference genome [25]. The primary procedures of NGS techniques are shown in Figure 1. which includes library fragmentation/amplicon generation along with integrated nucleotide determination. These techniques rely on various kinds of chemistries as well as base integration/detection devices. These are divided into two major types: techniques that employ PCR and those referred to as "single-molecule" sequencing approaches, that are not dependent on an amplification process [26, 27]. Upon the acquisition of the sequenced samples, similarity search is conducted utilising bioinformatic methods along with appropriate datasets and methods, including BLAST, NCBI, and others. In order to

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generate a new barcode and report an additional record for an already-existing barcode, adequate analysis of data is thought to be essential at that point [28]. A GenBank accession number is obtained for the sequence upon analytical authentication of the resemblance analysis [29]. In order to determine the species, data from the NCBI is then processed into the Barcode of Life Database [30].

Figure 1. Primary procedures of NGS technology.

3. Different Next Generation Sequencing Technologies

NGS refers to additional modern approaches, while the automated Sanger approach is regarded to be "first-generation" approach [31, 32]. Roche's 454 sequencing technology, which uses pyrosequencing—where the sequence is obtained by identifying the release of pyrophosphate when nucleotides are added to the DNA template—is one of the extensively utilised next generation sequencing technologies that has emerged [24]. Ion Torrent sequencing is an additional platform that uses the detection of hydrogen ion emission during DNA synthesis to ascertain the sequence. Reversible dye terminators serve as the foundation for the sequencing-by-synthesis technique used by the extensively used Illumina sequencing platform. SOLiD sequencing, or sequencing by oligonucleotide ligation and detection, is another emerging technology that uses a ligation-based strategy with reversible terminators to ascertain the DNA sequence. Numerous applications in genomics research are now possible due to the substantial productivity and speed gains of next-generation sequencing techniques [33]. The most recent development in the area of NGS is single-molecule sequencing. Longer read generation and remarkable data creation were combined by the single molecule sequencing (SMS) approach. It involves simultaneous identification of a single base at one time [34]. The very first commercialised platform built on the single-molecule sequencing concept was the Helicos genetic analysis system (Heliscope). Third-generation sequencing techniques have their roots in these advances in technology [10]. The general methodologies for various NGS technologies are shown in Figure 2.

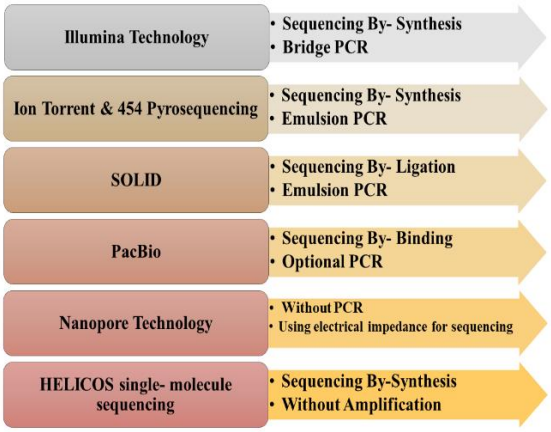

Figure 2. Methodologies for various NGS technologies.

Roche GS-FLX 454 genome sequencer

The first commercially viable next-generation sequencing technology, known as 454 pyrosequencing, was developed by 454 Life Sciences in 2005. Using sequencing chemistry, visible light is produced by an enzymatic system involving ATP sulfurylase, luciferase, and DNA polymerase, and this light is then detected and analysed [32]. This process measures the amount of pyrophosphate released during the continuous addition of nucleotides to the newly formed DNA strand. In 2005, the system was miniaturized and highly parallelized using Picotiter Plates, enabling it to generate over 200,000 reads with lengths of 100–150 bp each, resulting in a total output of 20 Mb per run [35]. Basic workflow of Roche 454- Pyrosequencing is shown in Figure 3.The 454 Sequencer, which produces 400–600 Mb of sequence reads each run and has the greatest number of short reads of any NGS platform at 600 bp, is essential for certain applications including RNA isoform detection in RNA-Seq and de novo assembly of microorganisms in metagenomics [36].When contrasted with competing NGS techniques, Roche closed out 454 Genome Sequencer around 2013 since its capabilities were no longer effective. As an outcome, the technique isn't used anymore for analysis with NGS and has been deemed outdated [11].

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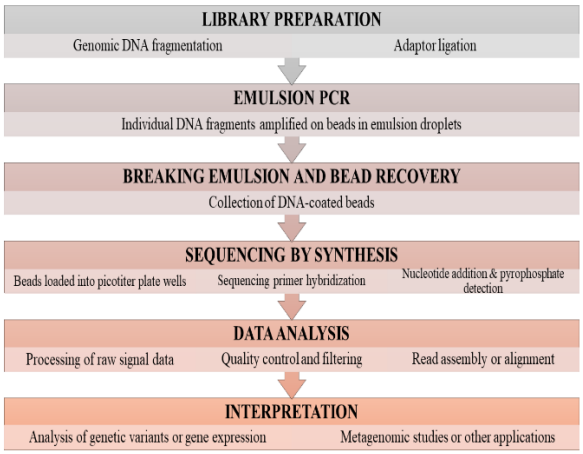

Figure 3. Workflow of Roche 454-Pyrosequencing.

Illumina/ Solexa genome analyzer

Currently the most extensively used technology, the Illumina/Solexa Genome Analyzer was the second platform to hit the market. Using the Illumina technology, sequencing-by synthesis is carried out by simultaneously adding all four nucleotides and DNA polymerase to oligo-primed cluster fragments in flow cell channels. For sequencing, bridge amplification lengthens cluster strands containing all four fluorescently labelled nucleotides. It's commonly acknowledged that the most flexible and user-friendly sequencing platform is the Genome Analyzer. Numerous genome sequencing studies have chosen it as their technology of choice due to its superior data quality and appropriate read lengths. The majority of NGS publications that have been published to date have explained techniques that make use of the short sequence data generated by the Genome Analyzer. Currently, the Illumina HiSeq 2000 Genome Analyzer can produce 200 giga base pairs (Gbp) of short sequences every run, and single read of up to 200 base pairs (pair-end reads). There is more than 99.5% correctness in the raw basis [37]. In contrast to the Roche 454 sequencer, the Illumina sequencer uses a technology called sequencing by synthesis, which uses detachable fluorescently labelled chain-terminating nucleotides to provide a higher yield at a cheaper cost of reagents [38]. The workflow of Illumina Sequencing technology is given in Figure 4.

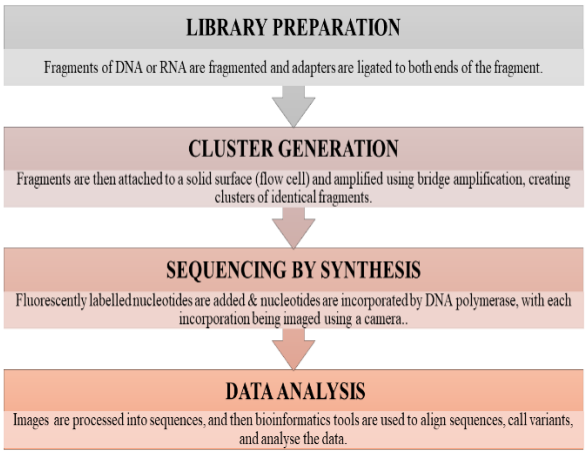

Figure 4. Workflow of Illumina Sequencing technology.

Ion torrent sequencing technology

Among the distinctive aspects of NGS technology is ion torrent, which does not depend on fluorescent dyes. This approach operates on the basis of detecting a shift in pH caused by Hydrogen ions released throughout nucleotide incorporation utilising the technique of semiconductors [39]. The workflow of ion torrent sequencing technology is shown in Figure 5. The nucleotides that are progressively added to the developing strand are detected by the system [11, 37]. Faster accumulation of sequencing information can be achieved using modest sample input using Ion Torrent Sequencing technologies, requiring a minimum of 24 hours and ten ng of input DNA. Furthermore, certain systems, like the Ion Chef system, enable automatic template along with library building. Quick determination as well as storage options are also provided by Ion Torrent sequencing technology [40].

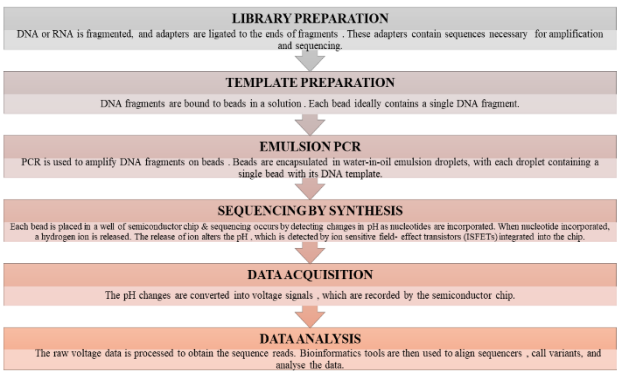

Figure 5. Workflow of Ion torrent sequencing technology

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Sequencing by Oligonucleotide Ligation and Detection (SOLiD)

The sequencing technology called SOLiD (Supported Oligonucleotide Ligation and Detection) was first launched by Applied Biosystems Instruments (ABI) in 2008 and currently marketed by Life Technologies. Two-nucleotide sequencing by ligation (SBL) serves as its foundation [32]. The process comprises ligating the probes after they have been successively annealed to the template. Current commercial sequencers, including the 5500 W series, are suitable for various scales of transcriptome, exome, and complete genome sequencing studies. Sample preparation and amplification in the past were comparable to Roche 454 sequencing. The sequencing procedures of the 5500 W series still use fluorescence-labelled octamer probes, involving multiple cycles of annealing and ligation. These cycles are analysed and decoded through a complex subtractive process, utilizing Exact Call Chemistry, which is extensively described by other researchers. [41]. This method has the advantage of precision because every base is questioned twice. The main drawbacks include the relatively extended run times of 7 to 14 days, short read lengths of 50–75 base pairs, and requirement for modern computer equipment and skilled computing personnel for raw data evaluation.

Third- generation sequencing technology

The desired DNA particles are attached to hairpin adapters on the two ends in PacBio Sequencing, including Sequel and RSII systems, leading to a closed, single-stranded circular DNA known as SMRT bell [42]. PacBio sequencing has an important feature over other NGS methods as it can generate extremely long reads. Hence this makes it the most suitable method for sequencing samples that are unidentified or libraries that include associated variations as it requires the fewest contigs to assemble lengthy read sequences. Furthermore, PacBio Sequencing has established itself as the industry standard for producing extremely accurate reference genomes through sequencing of the entire genome [40]. The workflow of PacBio sequencing technology is shown in Figure 6. In order to address the limitations of each technology alone, some researchers are employing hybrid sequencing procedures which combine PacBio sequencing with different NGS approaches [43].

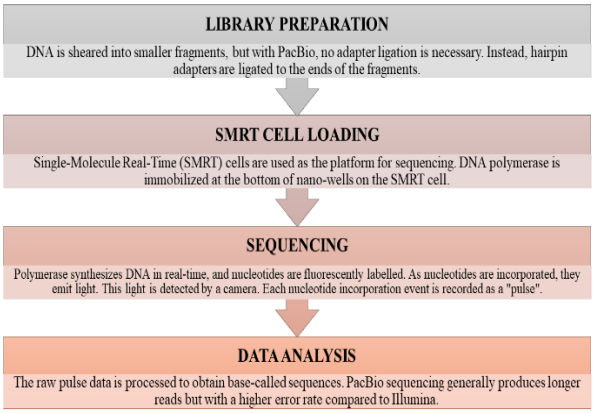

Figure 6. Workflow of PacBio sequencing technology.

Fourth-generation sequencing technology

Third-generation/single-molecule sequencing is coupled with nanopore technique in order to create fourth-generation sequencing [44]. A particular RNA or DNA molecule can be sequenced using this method without the need for amplification by PCR [19]. The implementation of nanopore sequencing technology could potentially be able to provide quick processing of samples alongside simultaneous outcomes presentation. It is an extremely versatile evaluation method as well as a cost-efficient genotyping approach [45]. Throughout the library preparation steps, the DNA molecules to be sequenced is attached with adaptor as well as processing enzymes like motor protein along with helicase. VolTRAX (Oxford Nanopore Technologies) provides an autonomous library preparation device that transforms biological samples into samples appropriate with Nanopore Sequencing investigation [40]. The framework of this technology which is invariably equipped by an electrolytic mechanism, exhibits voltage pass when an uninterrupted electrical field is given to it. The analysis of nanopore and the production of DNA or RNA which takes place within the nanopore determine the electrical current density across a nanopore interface [46]. In a study, the ONT Grid ION X5 technology was utilised to produce reads from the genomes of *S. barbata* D. Don and *Scutellaria baicalensis* [47]. Superior genome sequencing of *Gelsemium elegans* was accomplished in an investigation employing Hi-C and (ONT) Oxford Nanopore Sequencing Technology [48].

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4. Authentication Of Medicinal Herbs Or Plant-Based Products By Using Various Next Generation Sequencing Technologies

Several investigations on exploring the potential benefits of NGS for medicinal products authenticity as well as safety assurance are being reported. Cheng et al. used metagenomic research to identify the biological components of Liuwei Dihuang Wan, a traditional Chinese medicine [15]. Furthermore, it has been identified through results of NGS that there are notable differences in reliability and standard amongst producers and certain ones being impacted by biological contaminants. NGS was used by Coghlan et al. to identify the contents of 15 pharmaceutical goods that came in powdered form, pills, and tea-based forms [49]. Focusing the mitochondrial 16S ribosomal RNA gene as well as the plastid *trnL* gene's p-loop area, more than 49,000 amplification sequence readings have been generated, and 78% of the extracts had unlabelled taxa in their package. Additionally, research have been conducted on the implementation of PacBio RS II's SMRT sequencing technology for determining the constituents of ancient herbal mixtures Jiuwei Qianghuo Wan [50] as well as Danggui Buxue [51]. They enable consistent reads accessibility thus could be employed for quantifying since the quantity of input data is reflected in the total amount of sequences [52]. In this regard, Zhang et al. created the Danshen (*Salvia miltiorrhiza*) standard genomes using PacBio technologies [53]. The future potential of the technologies for molecular authentication of medicinal plants has been demonstrated by its application in the establishment of DNA markers for the authenticity of particular plant species, like *Boerhavia diffusa* and *Tinospora cordifolia* [54]. Because of the somewhat higher sequence reading errors of Nanopore Sequencing compared to other widely accessible NGS approaches, this method is yet to be utilised towards the verification of plant-based products.

Nanopore technology offers a potential tool for medicinal products, with improved reading quality [40]. Table 1 contains the illustrations of the utilisation of NGS techniques and Metabarcoding for the authentication of plant-based products [55- 81]. Liu et al. identified species in traditional herbal patent medicine, Wuhu San with the help of Illumina sequencing technology and shotgun metabarcoding [68]. Urumarudappa et al. conducted research on 39 Thai herbal products using ITS2 & *rbcL* markers and through next generation sequencing technology reveals the composition of several species [70]. Using Ion torrent sequencing technique and DNA metabarcoding, Seethapathy et al. authenticated Ayurvedic herbal products [71]. Another research conducted to identify endangered species in complex samples using cytochrome b, *matK*,16s, *rbcL*, mini-16S, COI, mini-COI, tRNA, intron, mini-cyt for the development and validation of multi-locus DNA Metabarcoding along with Illumina technology [72]. Speranskaya et al. done the comparative analysis of Illumina and Ion torrent High-throughput Sequencing technologies for identification of plant components in herbal teas [75]. Quality control of the traditional Chinese medicine, *Ruyi Jinhuang* with the help of Ion torrent technology and Real-Time PCR [76]. In another research Raclariu et al. identifies 16 *Veronica officinalis* therapeutic products [79], 53 *Echinacea* medicinal products [78] and 78 *Hypericum perforatum* drugs [80] by using NGS assisted metabarcoding technologies along with other chromatographic techniques. The traditional six herbal materials of Liuwei DihuangWan (LDW) a traditional Chinese medicine are reported as *C. officinalis, Alisma orientalis, Rehmannia glutinosa, Poria cocos, Paeonia suffruticosa.* Cheng et al. conducted the biological ingredient analysis of LDW via next generation sequencing technology [15].

5. Challenges

An improvement regarding sequencing output's read size (read lengths have become significantly lower across each newly introduced technologies) along with reliability (the modern technology’s bases are, on average, ten times less precise as compared to those acquired using Sanger sequencing).The read size, sequence dimension, sequence breadth and uniformity of coverage along with other factors impact the rate at which a DNA sequence generated from NGS reads and evaluation of the pipeline's reliability as well as the sequencing platform's efficiency [82, 83].

Another issue revolved around determining the overall output of the sequencing study relative to the expenditure and effort involved. Although there are many different

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Table 1 - Illustrations of the utilisation of NGS techniques & Metabarcoding for the Authentication of plant-based products.

NGS techniques	Plant-based Products	Marker used/ Amplified region	References
Shotgun-Metabarcoding	Four unlabelled plant species	*ITS2, psbA-trnH, matK, and rbcL*	[55]
DNA Metabarcoding, HPTLC	Two traditional Chinese herbal formulae	nrITS1 & nrITS2	[56]
NGS/Metabarcoding	17 single drugs and 15 polyherbal formulations	*rbcL* and ITS2	[57]
DNA Metabarcoding	*Lonicerae Japonicae* Flos (LJF)	ITS2	[58]
DNA Metabarcoding	*Lonicera* species	*psbA-trnH*	[59]
DNA Metabarcoding	Brahmi Herbal products	*rbcL* minibarcode	[60]
DNA Metabarcoding	*Chishao* species	*ycf1b* and *ndhF*	[61]
Illumina	Forty-seven plants and herb products	*matK, rbcL*, ITS2, *trnH-psbA, ropC, COI, rpoB, trnL-F, ycf1*	[62]
NGS/Metabarcoding	Herbal Teas	ITS2 & *psbA-trnH*	[63]
Shotgun Metabarcoding	Qingguo Wan	ITS2, *psbA-trnH*, *matK*, and *rbcL*	[64]
Metabarcoding	Ayurvedic polyherbal formulations	*rbcL*	[65]
Shotgun Metabarcoding	Fuke Desheng Wan (FKDSW)	ITS2, *matK* & *rbcL*	[66]
PCR and Metabarcoding	*Hedyotis* Herbal products	*trnL-trnF, psbA-trnH*	[67]
Illumina, Shotgun Metabarcoding	Wuhu San	ITS2, *psbA-trnH*, *matK*, and *rbcL*	[68]
NGS/Metabarcoding	Orchid along with other kinds of plants included in 55 commercial products	nrITS1 and nrITS2	[69]
NGS/Metabarcoding	39 Thai herbal products	ITS2 & *rbcL*	[70]
DNA Metabarcoding	*Senna obtusifolia (L.)*	*ycf1, rpl23, petL, and matK*	[28]

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Ion Torrent	Ayurvedic herbal products	nrITS1 & nrITS2	[71]
Illumina	18 traditional therapeutic products	Cytochrome b, *matK*,16s, *rbcL*, mini-16S, COI, mini-COI, tRNA, intron, mini-cyt	[72]
DNA metabarcoding	Commercial Plant products	*tnrL*	[73]
DNA Metabarcoding	*Hypericum perforatum L.*	ITS, ITS1 & ITS2	[74]
Illumina and Ion Torrent	Herbal Teas	nrITS1 and nrITS2	[75]
Ion Torrent	*Ruyi Jinhuang* powder	ITS2	[76]
Ion Torrent	*Echinacea* medicinal products	nrITS1 & nrITS2	[77]
PacBio	*Jiuwei Qianghuo Wan*	ITS2 & *psbA-trnH*	[50]
Illumina	Cobra Performance enhancer hard capsule	12 DNA barcode markers	[72]
Illumina	*Echinacea* species	Chloroplast genome	[78]
Ion Torrent	*Veronica officinalis* herbal products	nrITS1 & nrITS2	[79]
Ion Torrent	*Hypericum perforatum* herbal products	nrITS1 & nrITS2	[80]
Pac Bio	*Danggui Buxue*	ITS2 & *psbA-trnH*	[51]
NGS/Metabarcoding	17 plant species (Angiospermae & Cycadopsida)	*matK*, ITS2, *trnl*, *rbcl*, *psbA-trnH*	[72]
Ion Torrent	15 herbal supplements	*rbcL* & ITS2	[81]
PacBio	*Danshen*	Whole genome	[53]
454 Pyrosequencing	*C. officinalis, Alisma orientalis, Rehmannia glutinosa,Poria cocos,Paeonia suffruticosa*	*trnL* & ITS2	[15]
454 Pyrosequencing	Various species from 68 families	*trnL*	[49]

types of PCR bias in this additional problem, bias induced during amplification affects all PCR-based NGS technologies. Numerous investigations have demonstrated that higher template amounts, effective priming, by using varied replicate reaction arrangements, lower cycle numbers, lower annealing temperatures with careful selection, PCR bias is possible to be minimised greatly [84]. Recently NGS has been developed to encompass entire genome sequence and is regarded as an index measure of the number of repetitions a nucleotide was sequenced. The entire genome's sequences increased with an increase in

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the index. Because this method is so costly, it became essential to look for a different, more affordable method, such as Genome skimming. This technique is ideal for a decent assembling of repetitions, despite the low sequencing efficiency or modest sequence level. This method can generate around 1 gigabyte of sequences [85]. Because genome skimming is unable to identify uncommon varieties, it is comparable to Sanger sequencing [86]. The general expenses and effectiveness of NGS are increased employing selective DNA enrichment approaches which focus on particular areas that are of significance [87, 88]. However, consistent coverage, excellent repeatability, and absence of allele biases in any particular genomic area are necessary for focused enrichment [89].

6. DNA Metabarcoding with NGS

The accurate recognition of taxa in heterogeneous samples is made possible by metabarcoding technique, which has been developed through Next-generation sequencing technique [28]. DNA metabarcoding is the process of combining high-throughput sequencing with conventional DNA barcoding to enable simultaneous sequencing of the same barcode. DNA metabarcoding is being used to examine the degree of inconsistency between anticipated and identified plant species according to the marketing assertions of commercialised medicinal goods. It is useful for identifying varied plant species in a variety of goods [15, 49, 81, 79, 80]. The most significant benefit of DNA metabarcoding are its capacity to continuously detect individual species inside complicated mixtures containing several ingredients and processing steps, an area in which the applicability of both DNA barcoding and traditional analytical techniques is severely restricted. Because many factors significantly affect the acquired sequence read results, DNA metabarcoding data is only useful for qualitative evaluation—that is, for determining the presence of taxa—and not for quantitative estimation of relative number of species based on sequence read data [90]. This technique obtains the barcoded amplicon sequences by high-throughput sequencing technology and applies bioinformatic techniques to determine the species and variation within a sample [1]. De Boer conducted the research on Orchid along with other kinds of plants included in 55 commercial products using nrITS1 and nrITS2 through NGS technology along with Metabarcoding technique and reveals the widespread illegal trade of Orchid plant [69].

The usefulness and scientific depth of DNA barcoding is expanded by the recent advances in bioinformatics and molecular biology, which have made molecular data accessible from multiple databases. This technology allows simultaneous sequencing of multiple species in a single PCR run, thereby overcoming the effort-intensive species sorting and single species dependency that previously accompanied single species sequencing. In order to explore the diversity of species and determine the population density in a complex mixed community, metabarcoding allows multiplexing of hundreds of species from vast heterogeneous groups. This greatly boost detection procedures for proactive, cost-effective, and flexible management actions [91, 92]. For instance, in order to identify endangered plants in complicated samples, such as *Echinocactus*, *Euphorbia*, *Aloe variegate*, *Dendrobium*, *Cycas revolute*, and *Lactuca sativa*, Arulandhu developed a multi-locus DNA metabarcoding approach [72].

Using DNA metabarcoding in conjunction with other sequencing approaches, can recognise both authorised and adulterated Traditional Chinese Medicine (TCM) preparations like Jiuwei Qianghuo Wan [50] and Liuwei Dihuang Wan [15]. Certain illustrations of the utilisation of Metabarcoding for the authentication of plant-based products are given in Table 1. Nevertheless, a thorough and precise reference library of DNA sequences of a common genetic marker is necessary for the proper identification of species or herbal items using DNA metabarcoding technology [1].

7. Limitations Of DNA Metabarcoding

Similar to DNA barcoding, DNA metabarcoding has several restrictions. If there is any amplifiable DNA present, for example, these techniques may provide positive verification for herbal ingredients but false negative results may occur if the DNA has been damaged or deteriorated during afterwards processing or production [69]. DNA metabarcoding are only applicable for identifying and verifying processes in the context of the quality assurance of medicinal goods because they do not offer any quantitative or qualitative data regarding the metabolites that are active in the raw plant matter or the prepared substance.

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8. Recent NGS-Based Research on Therapeutic Plants

Using 454-GS FLX technology -based transcriptome sequencing, Gupta et al. revealed those genes that regulates production process of withanolides, which have therapeutic qualities from *Withania somnifera* L. Dunal [93]. *Phyllanthus amarus* Schum. and Thonn. leaf transcriptome study utilising the Illumina technology, investigated the roles of pathway-specific genes accountable for secondary metabolite biosynthesis [94]. In a different investigation, 454 pyrosequencing technique was used for determining thirty unique polymorphic microsatellite loci in *Ginkgo biloba* L. [95]. Using a transcriptomics approach based on Illumina Hiseq 2500 technology, researchers succeeded to identify genes associated with *Panax notoginseng* dormancy [96]. Jiuwei qianghuo wan (JWQHW), an 800-year-old traditional Chinese medicinal product containing 9 botanical components, was the goal of an investigation that using SMRT sequencing to identify some of its constituents [50]. Employing the PacBio RS II and Illumina Hiseq 2500 techniques, an experiment evaluated the genes associated with the production of phenolic acid in various *Salvia miltiorrhiza* root tissues [97]. Regarding the threatened herb *Aconitum austrokoreense*, Yun et al. employed Roche 454 GS-FLX Titanium technology to create nine microsatellite markers [98].

Luo et al. utilised the Roche's Titanium platform for determining the genes and transcriptome regulators intended towards the biosynthesis pathways in *Phlegmariurus carinatus* and *Huperzia serrat* [99]. An investigation into *Picrorhiza kurrooa*, 227 gigabytes of initial data from the Illumina and PacBio RS II technologies were used to build the initial genomes [100]. By applying a PacBio RS II device, Rui et al. carried out a transcriptome analysis of the Chinese therapeutic herb *Fritillaria cirrhosa* D. Don to identify genes associated with the production of iso-steroidal alkaloids [101]. Bhandari et al. identified a new microsatellite marker for *Salvadora oleoides* using paired-end Illumina sequencing technology [102].

Table 2 - Recent NGS technology - based research on therapeutic plants.

Different Species	NGS Technology used	Medicinal uses	Objectives	References
Gymnema Sylvestre	Illumina HiSeq 2500	Anti-diabetic properties	Genetic diversity assessment	[103]
Oregano Species	Metabarcoding and NMR	Antioxidant, antimicrobial, anti-inflammatory	Identify and quantify the level of adulteration in oregano species	[104]
Polygonati Rhizoma	Genome skimming	Blood sugar regulation, immune support	Testing and using complete plastomes for authentication of medicinal *Polygonatum* species	[105]
Coptis deltoidea	PacBio, Illumina technology	Carminative properties, anti-inflammatory, Anti-microbial	Analysis of genes that regulate the production of benzoquinoline	[106]

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			alkaloids	
Clitoria ternatea	Illumina technology	Anti-oxidant, Stress and anxiety relief	Finding potential genes related to the production of cyclotides	[107]
Aconitum carmichaellii	Illumina technology	Analgesic, Anti-inflammatory	Identify essential genes associated with the production of diterpene alkaloids.	[108]
Juglans regia	Illumina technology	Antioxidant, anti-diabetic	Gene coding for the biosynthesis of non-structural polyphenols	[109]
Panax ginseng	Illumina, Roche sequencing technologies	Anti-carcinogenic, Blood-sugar lowering, Neuro defensive	Recognition of cultivars along with investigation of genetic variation	[110, 111]
Ocimum tenuiflorum	Illumina sequencing technique	Antimicrobial, antidepressant	Genes behind strong therapeutic properties	[112]
Ocimum sanctum	Illumina HiSeq2000	Respiratory health, immune support, Anti-oxidant properties	Unravelling the genome of Holy basil: an "incomparable" "elixir of life" of traditional Indian medicine.	[113]
Pyrus communis	454-Pyrosequencing technology	Anti-bacterial, anti-fungal, Disinfectant	Construction along with description of the genome	[114]
Panax ginseng	Illumina HiSeq	Anticancer, to stimulate immune system	Transcriptome profiling and comparative analysis	[115]
Capsicum annum	Illumina technology	Acute diphtheria, flatulence, paralysis	Evolution, domestication and specialization studies	[116]

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Ziziphus jujuba	Illumina sequencing technique	Anti-bacterial, anti-inflammatory, wound healing properties	Vitamin C content, genomic resource	[117]
Catharanthus roseus	Illumina HiSeq 2000	Anticancer	Metabolic pathway production from RNA-Seq Data	[118]
Salvia miltiorrhiza	Illumina, Roche	Free radical scavenger, inflammation-reducing, Tumour - inhibiting	Finding of tanshinone biosynthesis-related genes	[119]
Nelumba nucifera	Illumina, 454-Pyrosequencing technology	Diarrhea, Anticancer	Genome characterization and origin	[120]
Curcuma longa	Illumina sequencing	Antitumor, anti-inflammatory	De novo transcriptome assembly	[121]
Allium sativum	GS FLX Titanium platform	Immunity booster, antimicrobial, anti-inflammatory	*De novo* assembly and characterization of transciptome	[122]
Azadirachta indica	Illumina, SMRT, PacBio-technologies	Anti-bacterial, anti-fungal, anti-viral	Terpenoid biosynthesis pathway	[123]
Phoenix dactylifera	Roche, SOLID	Oxidative stress reduction, cancer-fighting, gastric defence, Liver-protecting, Renal defence, immune system activation	Description as well as organisation of the genome and origin	[124, 125]
Prunus mume	Illumina technology	Antimicrobial, gastric ulcers	Genome characterization and evolution	[126]
Cannabis sativa	Illumina, Roche 454-Pyrosequencing technology	Hallucinogenic, Analgesic	Cannabinoid biosynthesis	[127]
Dendrobium officinale	Roche 454 GS FLX Titanium technique	Immunity booster, replenishment of fluids	Transcriptome analysis	[128]

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Bupleurum chinense	454 Pyrosequencing	Treat irregular menstruation, hepatitis	Biosynthesis of saikosaponins	[129]
Centella asiatica	Illumina	Diarrhea, fever, ulcers, relieves anxiety	Identification of the genes involved in primary and secondary metabolism	[130]
Withania somnifera	Pyrosequencing	Treatment of nervous and sexual disorders	Comparative analysis of leaf and root transcriptome for withanolide	[131]
Vasconcellea pubescens	Oxford Nanopore sequencing technology and Illumina technology	used to produce canned fruit, juice, jam and sweets	Comparative analysis of chloroplast genome	[132]
3 Korean Asarum species	Oxford Nanopore technology, Illumina	Aphthous stomatitis, toothache, and gingivitis	Chloroplast Genome Assembly	[133]
Chrysanthemum boreale	PacBio SMRT sequencing	White rust disease resistant, fever, inflammation, respiratory issues	Reconstruction of Chloroplast genome	[134]
Magnolia biondii	Oxford Nanopore sequencing technology	Treatment of allergic rhinitis and sinusitis	Mitochondrial genome assembly	[135]
Dalbergia odorifera	PacBio SMRT, Illumina technology	Treat blood stagnation syndrome, ischemia	Comparative analyses of genome structure and mitochondrial genome assembly	[136]
Sophora japonica	PacBio and Illumina technology	Anti-platelet, antioxidant, anti-inflammatory	Assembly and comparative analysis of the complete mitochondrial genome sequence	[137]
Rehmannia glutinosa	454 GS FLX Titanium	Constipation, Anaemia, dizziness	Genes involved in iridoid biosynthesis	[138]

Yoo et al. conducted research on 3 Korean Asarum species using Oxford nanopore technology and Illumina sequencing technology to determine the chloroplast genome assembly [133]. Another example of

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mitochondrial genome assembly is conducted by Dong et al. with the help of Oxford nanopore sequencing technology [135]. Comparative analysis of leaf and root transcriptome for withanolides biosynthetic pathways genes was determined by Gupta et al. with the help of Pyrosequencing technology [131]. Some of the other examples of recent NGS technology's – based research on therapeutic plants are given in Table 2.

9. Genome Skimming / Shotgun Metagenomics

Shotgun metagenomics often known as genome skimming refers to low-coverage sequencing of an entire genome. By using this method for plant-based goods, sequencing libraries can be produced despite the need for PCR amplification of barcode areas, consequently getting around PCR's restrictions in both DNA metabarcoding and traditional DNA barcoding. But in order to assemble a collection lacking PCR amplification, greater quantities of high-quality DNA required for being obtained from samples being tested, which can be difficult to get through extremely refined goods [8]. Sequences that comprise large repeat fractions of the genome such as inaccurate kilo base segments of the genomes of mitochondria, nuclear ribosomal DNA or whole plastid genomes may be obtained by genome skimming using a single-ingredient substance from plants [85]. Few papers have been published so far regarding utilisation of shotgun metagenomics for the recognition of plant-based goods. For the purpose to assess 20 widely accessible nutritional products of *Echinacea*, Handy et al. recently conducted genome skimming and DNA metabarcoding in conjunction with HPLC-UV assessment [139].

10. Discussion

According to some studies, whole genome sequencing could yield a higher species resolution than barcoding alone, hence the quick advancement of NGS could pose a danger to DNA barcoding research. However, genomic complexity, diversity, and function research is better served by whole genome sequencing as opposed to recognising species and biological monitoring [140]. Since whole plastid genome sequencing technology is advancing quickly, focus should be placed on which particular approach is appropriate for which species rather than on higher discriminating rates between species [141, 142, 84]. The dominant technique for NGS in the area of genomic studies has yet to be determined, it seems highly possible that NGS will ultimately become a crucial molecular method determining each area of biological studies due to ongoing cost savings, rapid enhancements in reliability and speed of sequencing [143].With the ability to sequence DNA at a speed that has never been achieved NGS technologies have opened up new avenues for biological research and previously unreachable advances in science. However, there is also a big problem with data storage, analysis and management due to the vast amount of data generated by NGS. The effective use of NGS technology requires sophisticated bioinformatics tools [143]. Metabarcoding is a technique that simultaneously identifies a variety of plants using global primers. Employing a metabarcoding approach, it has been demonstrated that it is possible to identify the presence of therapeutic herbs in manufactured medicinal products [144]. The years ahead will bring the massive manufacturing of phytomedicines as well as pharmaceutical innovation with discoveries made possible by useful and comparative genome sequencing techniques. The integration of metabolomics, proteomics, transcriptomics, and genomics is expected to offer investigators in the fields of phytopharmaceuticals, drug industry, preservation, systematic study, forensics analysis along with herbal industries a valuable means to comprehend phytomedicinal species from a systems biology standpoint [19].

11. Conclusion

In conclusion, the application of NGS for molecular authentication of medicinal plants has significantly advanced the field, offering a robust and reliable approach for ensuring the authenticity and quality of herbal medicines. Metagenomics and next-generation sequencing technology have made rapid advancements in DNA barcoding achievable. In herbal-based products, metabarcoding has shown possibilities as an approach to distinguish between tagged as well as unorganised varieties of plants. To get a high degree of accuracy, it is more satisfying to integrate taxonomic information with the DNA barcoding approach. Bioinformatics software is required during different stages of the barcoding processes to assist with gathering information, storage, evaluation, display, and appropriate management. NGS and DNA barcoding techniques is going to have a bright future owing to

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developments in bioinformatics. The integration of NGS into regulatory frameworks and quality control processes is essential for promoting consumer safety and confidence in the medicinal plant industry. In today's rapidly changing world, the significance of accurate authentication of medicinal plants cannot be overstated. By leveraging NGS, researchers can accurately identify plant species, detect adulterants and verify the botanical origin of medicinal plants. The findings underscore the importance of NGS in enhancing the safety and efficacy of herbal medicines. Moving forward, further research in NGS applications for plant authentication and the continuous development of NGS technologies are essential for advancing the field of molecular authentication of medicinal plants.

Author's Contribution

Both authors made significant contributions to the development and completion of this review article. JN: conducted an extensive literature search and drafted the sections of the review. VND: conceptualized the review, provided critical analysis and revised the content of this review article. Together, both authors collaborated on refining the manuscript, ensuring its accuracy and coherence. They have reviewed and approved the final version of the manuscript.

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Ethics Approval And Consent To Participate

This review article's information is drawn from studies that are widely accessible; the authors did not conduct any original research on humans or animals for this article. Therefore, consent for participation as well as approval of ethics are not essential.

Disclosure Statement

The authors affirm that they have no conflicts of interest to declare with respect to this manuscript.

References

1. Yu, J.; Wu, X.; Liu, C.; Newmaster, S.; Ragupathy, S.; Kress, W. J. Progress in the Use of DNA Barcodes in the Identification and Classification of Medicinal Plants. *Ecotoxicol. Environ. Saf.* **2021**, *208*, 111691.
2. Shen, Z.; Lu, T.; Zhang, Z.; Cai, C.; Yang, J.; Tian, B. Authentication of Traditional Chinese Medicinal Herb "Gusuibu" by DNA-Based Molecular Methods. *Ind. Crops Prod.* **2019**, *141*, 111756.
3. Niazian, M. Application of Genetics and Biotechnology for Improving Medicinal Plants. *Planta* **2019**, *249* (4), 953–973.
4. Heinrich, M.; Anagnostou, S. From Pharmacognosia to DNA-Based Medicinal Plant Authentication–Pharmacognosy Through the Centuries. *Planta Med.* **2017**, *83* (14/15), 1110–1116.
5. Chen, S.; Pang, X.; Song, J.; Shi, L.; Yao, H.; Han, J.; Leon, C. A Renaissance in Herbal Medicine Identification: From Morphology to DNA. *Biotechnol. Adv.* **2014**, *32* (7), 1237-1244.
6. Michel, C. I.; Meyer, R. S.; Taveras, Y.; Molina, J. The Nuclear Internal Transcribed Spacer (ITS2) as a Practical Plant DNA Barcode for Herbal Medicines. *J. Appl. Res. Med. Aromat. Plants* **2016**, *3* (3), 94–100.
7. Xiong, C.; Sun, W.; Li, J.; Yao, H.; Shi, Y.; Wang, P.; Chen, S. Identifying the species of seeds in traditional Chinese medicine using DNA barcoding. *Front. Pharmacol.* **2018**, *9*, 701.
8. Wu, H. Y.; Shaw, P. C. Strategies for molecular authentication of herbal products: from experimental design to data analysis. *Chin. Med.* **2022**, *17* (1), 38.
9. Parveen, I.; Gafner, S.; Techen, N.; Murch, S. J.; Khan, I. A. DNA Barcoding for the Identification of Botanicals in Herbal Medicine and Dietary Supplements: Strengths and Limitations. *Planta Med.* **2016**, *82* (14), 1225–1235
10. Sharma, S.; Shrivastava, N. Renaissance in Phytomedicines: Promising Implications of NGS Technologies. *Planta* **2016**, *244*, 19–38.
11. Das, S.; Mishra, R. Next Generation Sequencing Technologies Towards Exploration of Medicinal Plants.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 3110-3131 | ISSN:2251-6727

12. Gandhi, S. G.; Mahajan, V.; Bedi, Y. S. Changing Trends in Biotechnology of Secondary Metabolism in Medicinal and Aromatic Plants. *Planta* **2015**, *241*, 303-317.
13. Wang, A.; Wu, H.; Zhu, X.; Lin, J. Species identification of *Conyza bonariensis* assisted by chloroplast genome sequencing. *Front. Genet.* **2018**, *9*, 400591.
14. Meng, J.; Li, X.; Li, H.; Yang, J.; Wang, H.; He, J. Comparative Analysis of the Complete Chloroplast Genomes of Four *Aconitum* Medicinal Species. *Molecules* **2018**, *23* (5), 1015.
15. Cheng, X.; Su, X.; Chen, X.; Zhao, H.; Bo, C.; Xu, J.; Ning, K. Biological Ingredient Analysis of Traditional Chinese Medicine Preparation Based on High-Throughput Sequencing: The Story for Liuwei Dihuang Wan. *Sci. Rep.* **2014**, *4* (1), 5147.
16. Hu, L.; Hao, C.; Fan, R.; Wu, B.; Tan, L.; Wu, H. De Novo Assembly and Characterization of Fruit Transcriptome in Black Pepper (*Piper nigrum*). *PLoS One* **2015**, *10* (6), e0129822.
17. Liu, L.; Li, Y.; Li, S.; Hu, N.; He, Y.; Pong, R.; Law, M. Comparison of Next-Generation Sequencing Systems. *J. Biomed. Biotechnol.* **2012**, *2012*, 251364.
18. Li, F. S.; Weng, J. K. Demystifying Traditional Herbal Medicine with Modern Approach. *Nat. Plants* **2017**, *3* (8), 1–7.
19. Ganie, S. H.; Upadhyay, P.; Das, S.; Sharma, M. P. Authentication of Medicinal Plants by DNA Markers. *Plant Gene* **2015**, *4*, 83-99.
20. Singh, D.; Mathur, S.; Prasad, M.; Ranjan, R. Next-Generation Sequencing in Medicinal Plants: Recent Progress, Opportunities, and Challenges. *J. Plant Growth Regul.* **2024**, 1–17.
21. Sucher, N. J.; Hennell, J. R.; Carles, M. C. DNA fingerprinting, DNA barcoding, and next generation sequencing technology in plants. In *Plant DNA Fingerprinting and Barcoding: Methods and Protocols*; Humana Press: Totowa, NJ, **2012**; pp 13–22.
22. Haynes, E.; Jimenez, E.; Pardo, M. A.; Helyar, S. J. The Future of NGS (Next Generation Sequencing) Analysis in Testing Food Authenticity. *Food Control* **2019**, *101*, 134–143.
23. Prosser, S. W.; Hebert, P. D. Rapid Identification of the Botanical and Entomological Sources of Honey Using DNA Metabarcoding. *Food Chem.* **2017**, *214*, 183–191.
24. Satam, H.; Joshi, K.; Mangrolia, U.; Waghoo, S.; Zaidi, G.; Rawool, S.; Malonia, S. K. Next-Generation Sequencing Technology: Current Trends and Advancements. *Biology* **2023**, *12* (7), 997.
25. Behjati, S.; Tarpey, P. S. What Is Next Generation Sequencing? *Arch. Dis. Child. Educ. Pract. Ed.* **2013**, *98* (6), 236-238.
26. Shokralla, S.; Spall, J. L.; Gibson, J. F.; Hajibabaei, M. Next-Generation Sequencing Technologies for Environmental DNA Research. *Mol. Ecol.* **2012**, *21* (8), 1794–1805.
27. Grazina, L.; Amaral, J. S.; Mafra, I. Botanical Origin Authentication of Dietary Supplements by DNA-Based Approaches. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 1080–1109.
28. Yu, X.; Tan, W.; Gao, H.; Miao, L.; Tian, X. Development of a Specific Mini-Barcode from Plastome and Its Application for Qualitative and Quantitative Identification of Processed Herbal Products Using DNA Metabarcoding Technique: A Case Study on *Senna*. *Front. Pharmacol.* **2020**, *11*, 585687.
29. Clark, K.; Karsch-Mizrachi, I.; Lipman, D. J.; Ostell, J.; Sayers, E. W. GenBank. *Nucleic Acids Res.* **2016**, *44* (D1), D67-D72.
30. Ratnasingham, S.; Hebert, P. D. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Mol. Ecol. Notes* **2007**, *7* (3), 355–364.
31. Meyer, C. P.; Paulay, G. DNA Barcoding: Error Rates Based on Comprehensive Sampling. *PLoS Biol.* **2005**, *3* (12), e422.
32. Metzker, M. L. Sequencing Technologies—The Next Generation. *Nat. Rev. Genet.* **2010**, *11* (1), 31–46.
33. Pervez, M. T.; Abbas, S. H.; Moustafa, M. F.; Aslam, N.; Shah, S. S. M. A Comprehensive Review of Performance of Next-Generation Sequencing Platforms. *Biomed. Res. Int.* **2022**, *2022*.
34. Thompson, J. F.; Milos, P. M. The properties and applications of single-molecule DNA sequencing. *Genome Biol.* **2011**, *12*, 1–10.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 3110-3131 | ISSN:2251-6727

35. Mardis, E. R. Next-Generation DNA Sequencing Methods. *Annu. Rev. Genomics Hum. Genet.* **2008**, *9*, 387–402.
36. Mocali, S.; Benedetti, A. Exploring Research Frontiers in Microbiology: The Challenge of Metagenomics in Soil Microbiology. *Res. Microbiol.* **2010**, *161* (6), 497–505.
37. Zhang, J.; Chiodini, R.; Badr, A.; Zhang, G. The Impact of Next-Generation Sequencing on Genomics. *J. Genet. Genomics* **2011**, *38* (3), 95–109.
38. Shendure, J.; Ji, H. Next-Generation DNA Sequencing. *Nat. Biotechnol.* **2008**, *26* (10), 1135–1145.
39. Liu, L.; Li, Y.; Li, S.; Hu, N.; He, Y.; Pong, R.; Law, M. Comparison of Next-Generation Sequencing Systems. *J. Biomed. Biotechnol.* **2012**, *2012*, 251364.
40. Lo, Y. T.; Shaw, P. C. Application of Next-Generation Sequencing for the Identification of Herbal Products. *Biotechnol. Adv.* **2019**, *37* (8), 107450.
41. Gužvić, M. The History of DNA Sequencing. *J. Med. Biochem.* **2013**, *32* (4), 301–312.
42. Travers, K. J.; Chin, C. S.; Rank, D. R.; Eid, J. S.; Turner, S. W. A flexible and efficient template format for circular consensus sequencing and SNP detection. *Nucleic Acids Res.* **2010**, *38* (15), e159-e159.
43. Rhoads, A.; Au, K. F. PacBio Sequencing and Its Applications. *Genomics Proteomics Bioinformatics* **2015**, *13* (5), 278–289.
44. Singh, D.; Mittal, N.; Verma, S.; Singh, A.; Siddiqui, M. H. Applications of Some Advanced Sequencing, Analytical, and Computational Approaches in Medicinal Plant Research: A Review. *Mol. Biol. Rep.* **2024**, *51* (1), 23.
45. Senol Cali, D.; Kim, J. S.; Ghose, S.; Alkan, C.; Mutlu, O. Nanopore Sequencing Technology and Tools for Genome Assembly: Computational Analysis of the Current State, Bottlenecks and Future Directions. *Brief. Bioinform.* **2019**, *20* (4), 1542–1559.
46. Kumar, A.; Sharma, V. K.; Kumar, P. Nanopore Sequencing: The Fourth-Generation Sequencing. *J. Entomol. Zool. Stud.* **2019**, *7*, 1400–1403.
47. Xu, Z.; Gao, R.; Pu, X.; Xu, R.; Wang, J.; Zheng, S.; Song, J. Comparative genome analysis of *Scutellaria baicalensis* and *Scutellaria barbata* reveals the evolution of active flavonoid biosynthesis. *Genomics, Proteomics Bioinf.* **2020**, *18* (3), 230–240.
48. Liu, Y.; Tang, Q.; Cheng, P.; Zhu, M.; Zhang, H.; Liu, J.; Liu, Z. Whole-Genome Sequencing and Analysis of the Chinese Herbal Plant *Gelsemium elegans*. *Acta Pharm. Sin. B* **2020**, *10* (2), 374–382.
49. Coghlan, M. L.; Haile, J.; Houston, J.; Murray, D. C.; White, N. E.; Moolhuijzen, P.; Bunce, M. Deep Sequencing of Plant and Animal DNA Contained within Traditional Chinese Medicines Reveals Legality Issues and Health Safety Concerns. *PLoS Genet.* **2012**, *8* (4), e1002657.
50. Xin, T.; Xu, Z.; Jia, J.; Leon, C.; Hu, S.; Lin, Y.; Newmaster, S. G. Biomonitoring for traditional herbal medicinal products using DNA metabarcoding and single molecule, real-time sequencing. *Acta Pharm. Sin. B* **2018**, *8* (3), 488–497.
51. Zheng, X.; Zhang, P.; Liao, B.; Li, J.; Liu, X.; Shi, Y.; Chen, S. A Comprehensive Quality Evaluation System for Complex Herbal Medicine Using PacBio Sequencing, PCR-Denaturing Gradient Gel Electrophoresis, and Several Chemical Approaches. *Front. Plant Sci.* **2017**, *8*, 1578.
52. Williams, A.; Storton, D.; Buckles, J.; Llinas, M.; Wang, W. Improvement of PCR-free NGS library preparation to obtain uniform read coverage of genome with extremely high AT content. *J. Biomol. Tech.* **2012**, *23* (Suppl), S34.
53. Zhang, G.; Tian, Y.; Zhang, J.; Shu, L.; Yang, S.; Wang, W.; Chen, W. Hybrid de novo Genome Assembly of the Chinese Herbal Plant Danshen (*Salvia miltiorrhiza* Bunge). *Gigascience* **2015**, *4* (1), s13742-015.
54. Sharma, A. R.; Vohra, M.; Vinay, C. M.; Paul, B.; Chakrabarty, S.; Rai, P. S. Development of DNA Markers Using Next-Generation Sequencing Approach for Molecular Authentication of *Boerhavia diffusa* L. and *Tinospora cordifolia* (Willd.) Miers. *3 Biotech* **2023**, *13* (9), 304.
55. Zhang, Z.; Mu, W.; Kong, W.; Liu, J.; Zhao, J.; Zhao, Q.; Shi, L. Validation of the Shotgun Metabarcoding Approach for Comprehensively Identifying Herbal Products Containing Plant,

Fungal, and Animal Ingredients. *PLoS One* **2023**, *18* (7), e0286069.

56. Mück, F.; Scotti, F.; Mauvisseau, Q.; Raclariu-Manolică, A. C.; Schrøder-Nielsen, A.; Wangensteen, H.; De Boer, H. J. Complementary Authentication of Chinese Herbal Products to Treat Endometriosis Using DNA Metabarcoding and HPTLC Shows a High Level of Variability. *Front. Pharmacol.* **2023**, *14*, 1305410.
57. Travadi, T.; Shah, A. P.; Pandit, R.; Sharma, S.; Joshi, C.; Joshi, M. A combined approach of DNA metabarcoding collectively enhances the detection efficiency of medicinal plants in single and polyherbal formulations. *Front. Plant Sci.* **2023**, *14*, 1169984.
58. Dao, Y.; Yu, J.; Yang, M.; Han, J.; Fan, C.; Pang, X. DNA Metabarcoding Reveals the Fungal Community on the Surface of *Lonicerae Japonicae Flos*, an Edible and Medicinal Herb. *Int. J. Mol. Sci.* **2023**, *24* (20), 15081.
59. Gao, Y.; Zhang, X.; Wang, W.; Xing, Z.; Xu, L.; Tian, X. Qualitative Identification of *Lonicerae Japonicae Flos* in Traditional Chinese Medicine Using Metabarcoding Combined with Specific Mini-Barcodes. *Mol. Biol. Rep.* **2023**, *50* (11), 8817–8825.
60. Shah, A. P.; Travadi, T.; Sharma, S.; Pandit, R.; Joshi, C.; Joshi, M. Comprehensive Analysis Using DNA Metabarcoding, PCR, and HPLC Unveils the Adulteration in Brahmi Herbal Products. *bioRxiv* **2022**, *2022–07*.
61. Yang, X.; Yu, X.; Zhang, X.; Guo, H.; Xing, Z.; Xu, L.; Tian, X. Development of mini-barcode based on chloroplast genome and its application in metabarcoding molecular identification of Chinese medicinal material radix paeoniae rubra (Chishao). *Front. Plant Sci.* **2022**, *13*, 819822.
62. Delgado-Tejedor, A.; Leekitcharoenphon, P.; Aarestrup, F. M.; Otani, S. Evaluating the Usefulness of Next-Generation Sequencing for Herb Authentication. *Food Chem.: Mol. Sci.* **2021**, *3*, 100044.
63. Frigerio, J.; Agostinetto, G.; Mezzasalma, V.; De Mattia, F.; Labra, M.; Bruno, A. DNA-Based Herbal Teas' Authentication: An ITS2 and psbA-trnH Multi-Marker DNA Metabarcoding Approach. *Plants* **2021**, *10* (10), 2120.
64. Liu, J.; Shi, M.; Zhao, Q.; Kong, W.; Mu, W.; Xie, H.; Shi, L. Precise Species Detection in Traditional Herbal Patent Medicine, Qingguo Wan, Using Shotgun Metabarcoding. *Front. Pharmacol.* **2021**, *12*, 607210.
65. Pandit, R.; Travadi, T.; Sharma, S.; Joshi, C.; Joshi, M. DNA Meta-Barcoding Using rbcL Based Mini-Barcode Revealed Presence of Unspecified Plant Species in Ayurvedic Polyherbal Formulations. *Phytochem. Anal.* **2021**, *32* (5), 804–810.
66. Xie, H.; Zhao, Q.; Shi, M.; Kong, W.; Mu, W.; Li, B.; Shi, L. Biological ingredient analysis of Traditional Herbal Patent Medicine Fuke Desheng Wan using the shotgun metabarcoding approach. *Front. Pharmacol.* **2021**, *12*, 607197.
67. Yik, M. H. Y.; Lo, Y. T.; Lin, X.; Sun, W.; Chan, T. F.; Shaw, P. C. Authentication of *Hedyotis* Products by Adaptor Ligation-Mediated PCR and Metabarcoding. *J. Pharm. Biomed. Anal.* **2021**, *196*, 113920.
68. Liu, J.; Mu, W.; Shi, M.; Zhao, Q.; Kong, W.; Xie, H.; Shi, L. The Species Identification in Traditional Herbal Patent Medicine, Wuhu San, Based on Shotgun Metabarcoding. *Front. Pharmacol.* **2021**, *12*, 607200.
69. De Boer, H. J.; Ghorbani, A.; Manzanilla, V.; Raclariu, A. C.; Kreziou, A.; Ounjai, S.; Gravendeel, B. DNA Metabarcoding of Orchid-Derived Products Reveals Widespread Illegal Orchid Trade. *Proc. R. Soc. B* **2017**, *284* (1863), 20171182.
70. Urumarudappa, S. K. J.; Tungphatthong, C.; Prombutara, P.; Sukrong, S. DNA metabarcoding to unravel plant species composition in selected herbal medicines on the National List of Essential Medicines (NLEM) of Thailand. *Sci. Rep.* **2020**, *10*, 18259.
71. Seethapathy, G. S.; Raclariu-Manolica, A. C.; Anmarkrud, J. A.; Wangensteen, H.; de Boer, H. J. DNA Metabarcoding Authentication of Ayurvedic Herbal Products on the European Market Raises Concerns of Quality and Fidelity. *Front. Plant Sci.* **2019**, *10*, 68.
72. Arulandhu, A. J.; Staats, M.; Hagelaar, R.; Voorhuijzen, M. M.; Prins, T. W.; Scholtens, I.; Kok, E. Development and Validation of a Multi-Locus DNA Metabarcoding Method to Identify

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JCHR (2024) 14(3), 3110-3131 | ISSN:2251-6727

Endangered Species in Complex Samples. *GigaScience* **2017**, *6* (10), gix080.

73. Bruno, A.; Sandionigi, A.; Agostinetto, G.; Bernabovi, L.; Frigerio, J.; Casiraghi, M.; Labra, M. Food Tracking Perspective: DNA Metabarcoding to Identify Plant Composition in Complex and Processed Food Products. *Genes* **2019**, *10* (3), 248.
74. Howard, C.; Hill, E.; Kreuzer, M.; Mali, P.; Masiero, E.; Slater, A.; Sgamma, T. DNA Authentication of St John's Wort (*Hypericum perforatum* L.) Commercial Products Targeting the ITS Region. *Genes* **2019**, *10* (4), 286.
75. Speranskaya, A. S.; Khafizov, K.; Ayginin, A. A.; Krinitsina, A. A.; Omelchenko, D. O.; Nilova, M. V.; Logacheva, M. D. Comparative Analysis of Illumina and Ion Torrent High-Throughput Sequencing Platforms for Identification of Plant Components in Herbal Teas. *Food Control* **2018**, *93*, 315–324
76. Li, Q.; Sun, Y.; Guo, H.; Sang, F.; Ma, H.; Peng, H.; Xu, L. Quality Control of the Traditional Chinese Medicine Ruyi Jinhuang Powder Based on High-Throughput Sequencing and Real-Time PCR. *Sci. Rep.* **2018**, *8* (1), 8261.
77. Raclariu, A. C.; Ţebrencu, C. E.; Ichim, M. C.; Ciupercă, O. T.; Brysting, A. K.; de Boer, H. What's in the Box? Authentication of Echinacea Herbal Products Using DNA Metabarcoding and HPTLC. *Phytomedicine* **2018**, *44*, 32–38.
78. Zhang, N.; Erickson, D. L.; Ramachandran, P.; Ottesen, A. R.; Timme, R. E.; Funk, V. A.; et al. An Analysis of Echinacea Chloroplast Genomes: Implications for Future Botanical Identification. *Sci. Rep.* **2017**, *7*, 216. doi: 10.1038/s41598-017-00321-6.
79. Raclariu, A. C.; Mocan, A.; Popa, M. O.; Vlase, L.; Ichim, M. C.; Crisan, G.; de Boer, H. Veronica officinalis Product Authentication Using DNA Metabarcoding and HPLC-MS Reveals Widespread Adulteration with Veronica chamaedrys. *Front. Pharmacol.* **2017**, *8*, 378.
80. Raclariu, A. C.; Paltinean, R.; Vlase, L.; Labarre, A.; Manzanilla, V.; Ichim, M. C.; de Boer, H. Comparative Authentication of Hypericum perforatum Herbal Products Using DNA Metabarcoding, TLC and HPLC-MS. *Sci. Rep.* **2017**, *7* (1), 1291.
81. Ivanova, N. V.; Kuzmina, M. L.; Braukmann, T. W.; Borisenko, A. V.; Zakharov, E. V. Authentication of Herbal Supplements Using Next-Generation Sequencing. *PLoS One* **2016**, *11* (5), e0156426.
82. Sims, D.; Sudbery, I.; Ilott, N. E.; Heger, A.; Ponting, C. P. Sequencing Depth and Coverage: Key Considerations in Genomic Analyses. *Nat. Rev. Genet.* **2014**, *15* (2), 121–132.
83. Brozynska, M.; Furtado, A.; Henry, R. J. Direct Chloroplast Sequencing: Comparison of Sequencing Platforms and Analysis Tools for Whole Chloroplast Barcoding. *PLoS One* **2014**, *9* (10), e110387.
84. Ahmed, S. S. DNA Barcoding in Plants and Animals: A Critical Review. **2022**.
85. Straub, S. C.; Parks, M.; Weitemier, K.; Fishbein, M.; Cronn, R. C.; Liston, A. Navigating the tip of the genomic iceberg: Next-generation sequencing for plant systematics. *Am. J. Bot.* **2012**, *99* (2), 349–364.
86. Bakker, F. T.; Lei, D.; Yu, J.; Mohammadin, S.; Wei, Z.; van de Kerke, S.; Holmer, R. Herbarium Genomics: Plastome Sequence Assembly from a Range of Herbarium Specimens Using an Iterative Organelle Genome Assembly Pipeline. *Biol. J. Linn. Soc.* **2016**, *117* (1), 33-43.
87. Levin, J. Z.; Berger, M. F.; Adiconis, X.; Rogov, P.; Melnikov, A.; Fennell, T.; Gnirke, A. Targeted Next-Generation Sequencing of a Cancer Transcriptome Enhances Detection of Sequence Variants and Novel Fusion Transcripts. *Genome Biol.* **2009**, *10*, 1–8.
88. Rehman, A. U.; Morell, R. J.; Belyantseva, I. A.; Khan, S. Y.; Boger, E. T.; Shahzad, M.; Friedman, T. B. Targeted Capture and Next-Generation Sequencing Identifies C9orf75, Encoding Taperin, as the Mutated Gene in Nonsyndromic Deafness DFNB79. *Am. J. Hum. Genet.* **2010**, *86* (3), 378–388.
89. Stratton, M. Genome Resequencing and Genetic Variation. *Nat. Biotechnol.* **2008**, *26* (1), 65-66.
90. Staats, M.; Arulandhu, A. J.; Gravendeel, B.; Holst-Jensen, A.; Scholtens, I.; Peelen, T.; Kok, E. Advances in DNA Metabarcoding for Food and Wildlife Forensic Species Identification. *Anal. Bioanal. Chem.* **2016**, *408*, 4615–4630.

91. Piper, A. M.; Batovska, J.; Cogan, N. O.; Weiss, J.; Cunningham, J. P.; Rodoni, B. C.; Blacket, M. J. Prospects and Challenges of Implementing DNA Metabarcoding for High-Throughput Insect Surveillance. *GigaScience* **2019**, *8* (8), giz092.
92. Gao, Z.; Liu, Y.; Wang, X.; Wei, X.; Han, J. DNA Mini-Barcoding: A Derived Barcoding Method for Herbal Molecular Identification. *Front. Plant Sci.* **2019**, *10*, 417036.
93. Gupta, P.; Goel, R.; Agarwal, A. V.; Asif, M. H.; Sangwan, N. S.; Sangwan, R. S.; Trivedi, P. K. Comparative Transcriptome Analysis of Different Chemotypes Elucidates Withanolide Biosynthesis Pathway from Medicinal Plant *Withania somnifera*. *Sci. Rep.* **2015**, *5* (1), 18611.
94. Bose Mazumdar, A.; Chattopadhyay, S. Sequencing, De Novo Assembly, Functional Annotation and Analysis of Phyllanthus amarus Leaf Transcriptome Using the Illumina Platform. *Front. Plant Sci.* **2016**, *6*, 162661.
95. Zhou, Q.; Wang, X. X.; Xu, M.; Cao, F. L.; Yu, F. X.; Xu, L. A. Development and Characterization of Novel Microsatellite Markers for *Ginkgo biloba* Using 454 Pyrosequencing. *Genet Mol Res* **2016**.
96. Yang, K.; Yang, L.; Fan, W.; Long, G. Q.; Xie, S. Q.; Meng, Z. G.; Chen, J. W. Illumina-based transcriptomic analysis on recalcitrant seeds of *Panax notoginseng* for the dormancy release during the after-ripening process. *Physiol. Plant.* **2019**, *167* (4), 597–612.
97. Xu, Z.; Luo, H.; Ji, A.; Zhang, X.; Song, J.; Chen, S. Global identification of the full-length transcripts and alternative splicing related to phenolic acid biosynthetic genes in *Salvia miltiorrhiza*. *Front. Plant Sci.* **2016**, *7*, 100.
98. Yun, Y. E.; Yu, J. N.; Nam, G. H.; Ryu, S. A.; Kim, S.; Oh, K.; Lim, C. E. Next-Generation Sequencing Identification and Characterization of Microsatellite Markers in *Aconitum austrokoreense* Koidz., an Endemic and Endangered Medicinal Plant of Korea. *Genet. Mol. Res.* **2015**, *14* (2), 4812–4817.
99. Luo, H.; Li, Y.; Sun, C.; Wu, Q.; Song, J.; Sun, Y.; Chen, S. Comparison of 454-ESTs from *Huperzia serrata* and *Phlegmariurus carinatus* Reveals Putative Genes Involved in Lycopodium Alkaloid Biosynthesis and Developmental Regulation. *BMC Plant Biol.* **2010**, *10*, 1–16.
100. Sharma, T.; Sharma, N. K.; Kumar, P.; Panzade, G.; Rana, T.; Swarnkar, M. K.; Kumar, S. The First Draft Genome of Picrorhiza kurrooa, an Endangered Medicinal Herb from Himalayas. *Sci. Rep.* **2021**, *11* (1), 14944.
101. Li, R.; Xiao, M.; Li, J.; Zhao, Q.; Wang, M.; Zhu, Z. Transcriptome Analysis of CYP450 Family Members in *Fritillaria cirrhosa* D. Don and Profiling of Key CYP450s Related to Isosteroidal Alkaloid Biosynthesis. *Genes* **2023**, *14* (1), 219.
102. Bhandari, M. S.; Meena, R. K.; Shamoon, A.; Saroj, S.; Kant, R.; Pandey, S. First De Novo Genome Specific Development, Characterization and Validation of Simple Sequence Repeat (SSR) Markers in Genus Salvadora. *Mol. Biol. Rep.* **2020**, *47* (9), 6997-7008.
103. Polaiah, A. C.; Damor, P. R.; Reddy, R. N.; Manivel, P.; Shivakumara, K. T.; Suthar, M. K.; Kumar, J. Development of Genomic SSR Markers in Gymnema sylvestre (Retz.) R. Br. ex Sm. Using Next Generation DNA Sequencing and Their Application in Genetic Diversity Analysis. *J. Appl. Res. Med. Aromat. Plants* **2023**, *34*, 100455.
104. Flügge, F.; Kerkow, T.; Kowalski, P.; Bornhöft, J.; Seemann, E.; Creydt, M.; Günther, U. L. Qualitative and Quantitative Food Authentication of Oregano Using NGS and NMR with Chemometrics. *Food Control* **2023**, *145*, 109497.
105. Wang, S.; Zhou, N.; Shi, N.; Zhang, G.; Liu, H.; Guo, X.; Ji, Y. Testing and using complete plastomes for authentication of medicinal *Polygonatum* species (*Asparagaceae*). *Ind. Crops Prod.* **2023**, *197*, 116557.
106. Zhong, F.; Huang, L.; Qi, L.; Ma, Y.; Yan, Z. Full-Length Transcriptome Analysis of *Coptis deltoidea* and Identification of Putative Genes Involved in Benzylisoquinoline Alkaloids Biosynthesis Based on Combined Sequencing Platforms. *Plant Mol. Biol.* **2020**, *102*, 477–499.
107. Kalmankar, N. V.; Venkatesan, R.; Balaram, P.; Sowdhamini, R. Transcriptomic Profiling of the Medicinal Plant *Clitoria ternatea*: Identification of Potential Genes in Cyclotide Biosynthesis. *Sci. Rep.* **2020**, *10* (1), 12658.
108. Rai, A.; Saito, K.; Yamazaki, M. Integrated Omics Analysis of Specialized Metabolism in Medicinal Plants. *Plant J.* **2017**, *90* (4), 764–787.

109. Martínez-García, P. J.; Crepeau, M. W.; Puiu, D.; Gonzalez-Ibeas, D.; Whalen, J.; Stevens, K. A.; Neale, D. B. The Walnut (*Juglans regia*) Genome Sequence Reveals Diversity in Genes Coding for the Biosynthesis of Non-Structural Polyphenols. *Plant J.* **2016**, *87* (5), 507–532.
110. Li, M. R.; Shi, F. X.; Zhou, Y. X.; Li, Y. L.; Wang, X. F.; Zhang, C.; Li, L. F. Genetic and Epigenetic Diversities Shed Light on Domestication of Cultivated Ginseng (*Panax ginseng*). *Mol. Plant* **2015**, *8* (11), 1612–1622.
111. Zhao, Y.; Yin, J.; Guo, H.; Zhang, Y.; Xiao, W.; Sun, C.; Xiao, J. The Complete Chloroplast Genome Provides Insight into the Evolution and Polymorphism of *Panax ginseng*. *Front. Plant Sci.* **2015**, *5*, 696.
112. Upadhyay, A. K.; Chacko, A. R.; Gandhimathi, A.; Ghosh, P.; Harini, K.; Joseph, A. P.; Sowdhamini, R. Genome sequencing of herb Tulsi (*Ocimum tenuiflorum*) unravels key genes behind its strong medicinal properties. *BMC Plant Biol.* **2015**, *15*, 1–20.
113. Rastogi, S.; Kalra, A.; Gupta, V.; Khan, F.; Lal, R. K.; Tripathi, A. K.; Shasany, A. K. Unravelling the Genome of Holy Basil: An "Incomparable" "Elixir of Life" of Traditional Indian Medicine. *BMC Genomics* **2015**, *16*, 1–15.
114. Chagné, D.; Crowhurst, R. N.; Pindo, M.; Thrimawithana, A.; Deng, C.; Ireland, H.; Velasco, R. The Draft Genome Sequence of European Pear (*Pyrus communis* L. 'Bartlett'). *PLoS One* **2014**, *9* (4), e92644.
115. Jayakodi, M.; Lee, S. C.; Park, H. S.; Jang, W.; Lee, Y. S.; Choi, B. S.; Yang, T. J. Transcriptome Profiling and Comparative Analysis of *Panax ginseng* Adventitious Roots. *J. Ginseng Res.* **2014**, *38* (4), 278–288.
116. Qin, C.; Yu, C.; Shen, Y.; Fang, X.; Chen, L.; Min, J.; Zhang, Z. Whole-Genome Sequencing of Cultivated and Wild Peppers Provides Insights into Capsicum Domestication and Specialization. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111* (14), 5135–5140.
117. Liu, M. J.; Zhao, J.; Cai, Q. L.; Liu, G. C.; Wang, J. R.; Zhao, Z. H.; Luo, L. H. The Complex Jujube Genome Provides Insights into Fruit Tree Biology. *Nat. Commun.* **2014**, *5* (1), 5315.
118. Van Moerkercke, A.; Fabris, M.; Pollier, J.; Baart, G. J.; Rombauts, S.; Hasnain, G.; Goossens, A. CathaCyc, a metabolic pathway database built from Catharanthus roseus RNA-Seq data. *Plant Cell Physiol.* **2013**, *54* (5), 673–685.
119. Yang, L.; Ding, G.; Lin, H.; Cheng, H.; Kong, Y.; Wei, Y.; Yang, C. Transcriptome analysis of medicinal plant *Salvia miltiorrhiza* and identification of genes related to tanshinone biosynthesis. *PLoS One* **2013**, *8* (11), e80464.
120. Ming, R.; VanBuren, R.; Liu, Y.; Yang, M.; Han, Y.; Li, L. T.; Shen-Miller, J. Genome of the Long-Living Sacred Lotus (*Nelumbo nucifera* Gaertn.). *Genome Biol.* **2013**, *14*, 1–11.
121. Annadurai, R. S.; Neethiraj, R.; Jayakumar, V.; Damodaran, A. C.; Rao, S. N.; Katta, M. A.; Mugasimangalam, R. C. De Novo Transcriptome Assembly (NGS) of Curcuma longa L. Rhizome Reveals Novel Transcripts Related to Anticancer and Antimalarial Terpenoids. *PLoS One* **2013**, *8* (2), e56217.
122. Sun, X.; Zhou, S.; Meng, F.; Liu, S. De novo assembly and characterization of the garlic (*Allium sativum*) bud transcriptome by Illumina sequencing. *Plant Cell Rep.* **2012**, *31*, 1823–1828.
123. Krishnan, N. M.; Pattnaik, S.; Jain, P.; Gaur, P.; Choudhary, R.; Vaidyanathan, S.; Panda, B. A Draft of the Genome and Four Transcriptomes of a Medicinal and Pesticidal Angiosperm *Azadirachta indica*. *BMC Genomics* **2012**, *13*, 1–13.
124. Fang, Y.; Wu, H.; Zhang, T.; Yang, M.; Yin, Y.; Pan, L.; Yu, J. A Complete Sequence and Transcriptomic Analyses of Date Palm (*Phoenix dactylifera* L.) Mitochondrial Genome. *PLoS One* **2012**, *7* (5), e37164.
125. Yang, M.; Zhang, X.; Liu, G.; Yin, Y.; Chen, K.; Yun, Q.; Yu, J. The complete chloroplast genome sequence of date palm (*Phoenix dactylifera* L.). *PLoS One* **2010**, *5* (9), e12762.
126. Zhang, Q.; Chen, W.; Sun, L.; Zhao, F.; Huang, B.; Yang, W.; Wang, J. The Genome of *Prunus mume*. *Nat. Commun.* **2012**, *3* (1), 1318.
127. Van Bakel, H.; Stout, J. M.; Cote, A. G.; Tallon, C. M.; Sharpe, A. G.; Hughes, T. R.; Page, J. E. The draft genome and transcriptome of Cannabis sativa. *Genome Biol.* **2011**, *12*, 1–18.
128. Guo, X.; Li, Y.; Li, C.; Luo, H.; Wang, L.; Qian, J.; Chen, S. Analysis of the *Dendrobium officinale*

Transcriptome Reveals Putative Alkaloid Biosynthetic Genes and Genetic Markers. *Gene* **2013**, *527* (1), 131-138.

129. Sui, C.; Zhang, J.; Wei, J.; Chen, S.; Li, Y.; Xu, J.; Xu, Y. Transcriptome Analysis of Bupleurum chinense Focusing on Genes Involved in the Biosynthesis of Saikosaponins. *BMC Genomics* **2011**, *12*, 1-16.
130. Sangwan, R. S.; Tripathi, S.; Singh, J.; Narnoliya, L. K.; Sangwan, N. S. De Novo Sequencing and Assembly of Centella asiatica Leaf Transcriptome for Mapping of Structural, Functional and Regulatory Genes with Special Reference to Secondary Metabolism. *Gene* **2013**, *525* (1), 58-76.
131. Gupta, P.; Goel, R.; Pathak, S.; et al. De Novo Assembly, Functional Annotation and Comparative Analysis of *Withania somnifera* Leaf and Root Transcriptomes to Identify Putative Genes Involved in the Withanolides Biosynthesis. *PLoS One* **2013**, *8*, 62714.
132. Lin, Z.; Zhou, P.; Ma, X.; Deng, Y.; Liao, Z.; Li, R.; Ming, R. Comparative Analysis of Chloroplast Genomes in Vasconcellea pubescens A. DC. and Carica papaya L. *Sci. Rep.* **2020**, *10* (1), 15799.
133. Yoo, M. J.; Jin, D. P.; Lee, H. O.; Lim, C. E. Complete Plastome of Three Korean Asarum (Aristolochiaceae): Confirmation Tripartite Structure within Korean Asarum and Comparative Analyses. *Plants* **2021**, *10* (10), 2056.
134. Won, S. Y.; Jung, J. A.; Kim, J. S. The Complete Chloroplast Genome of Chrysanthemum boreale (Asteraceae). *Mitochondrial DNA Part B* **2018**, *3* (2), 549-550.
135. Dong, S.; Chen, L.; Liu, Y.; Wang, Y.; Zhang, S.; Yang, L.; Zhang, S. The Draft Mitochondrial Genome of Magnolia biondii and Mitochondrial Phylogenomics of Angiosperms. *PLoS One* **2020**, *15* (4), e0231020.
136. Hong, Z.; Liao, X.; Ye, Y.; Zhang, N.; Yang, Z.; Zhu, W.; Wu, Z. A Complete Mitochondrial Genome for Fragrant Chinese Rosewood (Dalbergia odorifera, Fabaceae) with Comparative Analyses of Genome Structure and Intergenomic Sequence Transfers. *BMC Genomics* **2021**, *22*, 1-13.
137. Shi, Y.; Liu, Y.; Zhang, S.; Zou, R.; Tang, J.; Mu, W.; Dong, S. Assembly and Comparative Analysis of the Complete Mitochondrial Genome Sequence of Sophora japonica 'JinhuaiJ2'. *PLoS One* **2018**, *13* (8), e0202485.
138. Sun, P.; Song, S.; Zhou, L.; Zhang, B.; Qi, J.; Li, X. Transcriptome Analysis Reveals Putative Genes Involved in Iridoid Biosynthesis in Rehmannia glutinosa. *Int. J. Mol. Sci.* **2012**, *13* (10), 13748-13763.
139. Handy, S. M.; Pawar, R. S.; Ottesen, A. R.; Ramachandran, P.; Sagi, S.; Zhang, N.; Erickson, D. L. HPLC-UV, Metabarcoding and Genome Skims of Botanical Dietary Supplements: A Case Study in Echinacea. *Planta Med.* **2021**, *87* (4), 314–324.
140. Grant, D. M.; Brodnicke, O. B.; Evankow, A. M.; Ferreira, A. O.; Fontes, J. T.; Hansen, A. K.; Ekrem, T. The Future of DNA Barcoding: Reflections from Early Career Researchers. *Diversity* **2021**, *13* (7), 313.
141. Li, X.; Yang, Y.; Henry, R. J.; Rossetto, M.; Wang, Y.; Chen, S. Plant DNA Barcoding: From Gene to Genome. *Biol. Rev.* **2015**, *90* (1), 157–166.
142. Liu, Z. F.; Ma, H.; Ci, X. Q.; Li, L.; Song, Y.; Liu, B.; Li, J. Can Plastid Genome Sequencing Be Used for Species Identification in Lauraceae? *Bot. J. Linn. Soc.* **2021**, *197* (1), 1–14.
143. Zhang, J.; Chiodini, R.; Badr, A.; Zhang, G. The Impact of Next-Generation Sequencing on Genomics. *J. Genet. Genomics* **2011**, *38* (3), 95–109.
144. Gesto-Borroto, R.; Medina-Jiménez, K.; Lorence, A.; Villarreal, M. L. Application of DNA Barcoding for Quality Control of Herbal Drugs and Their Phytopharmaceuticals. *Rev. Bras. Farmacogn.* **2021**, *31*, 127–141.