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Molecular pharmacognosy

HUANG LuQi[1*], XIAO PeiGen[2], GUO LanPing[1] & GAO WenYuan[3]

[1]*Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China;*
[2]*Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100094, China;*
[3]*School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, China*

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This article analyzes the background and significance of molecular pharmacognosy, including the molecular identification of medicinal raw materials, phylogenetic evolution of medicinal plants and animals, evaluation and preservation of germplasm resources for medicinal plants and animals, etiology of endangerment and protection of endangered medicinal plants and animals, biosynthesis and bioregulation of active components in medicinal plants, and characteristics and the molecular bases of top-geoherbs.

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Molecular pharmacognosy is a science that studies the classification, identification, cultivation and conservation of medicinal raw materials, as well as the production of their active components at a molecular level [1]. The concept of "molecular pharmacognosy" was first introduced by HUANG *et al*. [2] in *Prospects for Application of Molecular Biotechnique to Pharmacognosy* in 1995. In June 2000, The Press of Beijing Medical University published *Molecular Pharmacognosy* [3]. The second edition of the book appeared in 2006 [4], and the same year *Molecular Pharmacognosy* was listed among teaching materials for undergraduates [1]. Molecular pharmacognosy has been included in the curriculum of many traditional Chinese medicine colleges and medical universities. This paper introduces the background, development, and significance of this science, reviews its advances in the last decade, and discusses the direction of its future progress.

1 Background of the emergence of molecular pharmacognosy

1.1 Concepts of medicinal raw material and pharmacognosy

Medicinal raw materials are derived from natural medicinal materials, including fresh or simply-processed plants, animals and minerals which are intended for applications in disease treatment, health-care or the production of medicines [5]. Medicinal plants and animals are the main object of pharmacognosy, accounting for up to 99% of all medicinal raw materials. The term "shengyao", sometimes translated as "medicinal raw material", but more precisely meaning "unrefined substance", was initially found in the regulations of the Imperial Medical Academy of the Ming Dynasty. A sentence from those regulations stipulated that "[A sample of] all kinds of medicinal materials under the sun must be preserved in our academy's drug storage place for Medicinal Raw Materials, and all medicines prescribed

*Corresponding author (email: huangluqi@263.net)

in the academy must be processed on the basis of those medicinal raw materials that are collected from different places" [6]. Encompassing applications of herbal literature resources, botany, zoology, chemistry (including phytochemistry, analytical chemistry and biochemistry), pharmacology, traditional Chinese medicine, clinical medicine and molecular biology as well as modern technologies, pharmacognosy essentially studies the origin, identification, active components, production, collection, and quality evaluation of medicinal raw materials, as well as their sustainable development and utilization [7]. The term "pharmacognosy" was initially used in 1880 by Oi GenDo, in one of his translations entitled *Pharmacognosy* [7]. A survey of pharmacognosy shows that the focus in ancient times was mostly restricted to herbal literature, while contemporary connotations and denotations of the pharmacognosy may overlap with those of Traditional Chinese Medicinal (TCM) resourceology and science of TCM identification.

1.2 Achievements in pharmacognosy research and development

Significant progress has been made in both investigative and applied pharmacognosy, in the exploration, collection, enlargement, conservation and utilization of resources, in systematics, and in the quality control of commonly-used TCM. A comprehensive picture of all TCM species has been obtained by three rounds of nationwide investigations of TCM resources, with details of each species' distribution, ecological environment, reservations, historical evolution, production, and experience acquired from traditional utilization. The systematics of 220 commonly-used TCM species has been methodically studied. Quality standards for 71 TCM species have been established. Chemical studies has been conducted on 400 different kinds of TCM materials, and over 800 biologically-active components have been isolated, thereby identifying the chemical contents of many medicinal herbs. In addition, such Chinese herbal species as *Styrax macrothyrsus*, *S. subniveus,* and *S. hypoglauca* (Anxixiang) were determined to be alternative resources for imported herbs. Artificial products and substitutes have been developed for such medicinal herbs as *Calculus Bovis* (Niuhuang), *Moschus* (Shexiang), *Os Tigris* (Hugu), *Cornu Rhinocerotis* (Xijiao), and *Cordyceps sinensis* (Dongchongxiacao). The medicinal uses of the different parts of each TCM species have been thoroughly evaluated. Stalks of *Uncaria rhynchophylla* (Gouteng) were added as a new medicinal part of the herb in addition to its hooks [8]. Artificial propagation and field-cultivation of medicinal plants, and medicinal animal breeding have undergone significant development. Good agricultural practice (GAP) for TCM has been implemented in China since 1999, and the pressure on wild TCM resources has been significantly reduced. A number of major reference books have been published, including: *Compilation of Chinese Medicinal Herbs*, *Dictionary of Traditional Chinese Medicine*, *Xinhua Compendium of Chinese Herbology*, *Traditional Chinese Medicinal Resources* and *Classes of Traditional Chinese Medicine* [9].

1.3 Problems and limitations in pharmacognosy research

As a growing and diversifying subject, pharmacognosy has to face its own limitations in the areas of investigation and techniques. For example, techniques used for the identification of medicinal materials, which is the core of pharmacognosy and the foundation for species systematics, resource investigations, conservation and the utilization of medicinal materials, have identified counterfeit substances from genuine medicinal materials, initially on the basis of external morphological characteristics, such as color, luster, fracture surfaces, texture and odor. More techniques were later developed, such as microscopic identification of internal cellular and histological structures, scanning electron microscopy for observation of ultrastructures, and physicochemical identification based on physicochemical characteristics of medicinal materials. Pharmacognosy also utilizes spectroscopic analytical techniques [10]. However, a number of unresolved problems impede the identification of medicinal raw materials. For example, animal medicinal materials cannot be effectively identified because of their unclear active components and implicit characteristics. To cite another example, counterfeits of some valuable medicinal materials are available for purchase due to the lack of effective identification methods. Because medicinal materials originate from various plants, counterfeit components pose problems which impact the stability and homogeneity of TCM quality. It is still popular that one TCM has multi-origins, for example, the roots from three plants, including *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao and *A. membranaceus* (Fisch.) Bge could be utilized as drug *Radix Astragtali* (Huangqi), while both roots from *Glycyrrhiza uralensis* Fisch., *G. inflata* Bat., and *G. glabra* L are used as drug Radix et Rhizoma Glycyrrhizae (Gancao) [11].

Biologically speaking, every species has its own hereditary characteristics and phenotype, as well as its specific way to adapt to a new environment, thus medicinal materials from different origins do not comprise a single medicine. For this reason, to establish correlations between biological species and TCM species, it was decided that TCM species should be divided or combined according to their different origins in the *China Pharmacopoeia 2005 Edition*. However, this plan failed to materialize because of discrepancies in opinions as to the evolutionary classification of TCM species, the uncertain systemic relationships, and the scarcity of genetic evidence for ascertaining their origins. The multi-origin of TCM remains a problem for future editions of *China Pharmacopoeia*. For example, the rhizomes from

two *Atractylodis* plants can be utilized as drug *Rhizoma Atractylodis* (Cangzhu), while the flowers from three *Lonicera* plants can be collected as the drug *Flos Lonicerae* (Shanyinhua). The development of pharmacognosy has led to expectations that new theories, methods and techniques will result in further advances in this field.

1.4 Studies at the molecular level: Essential for the development of pharmacognosy

Developments in science and technology, especially in biology and related subjects, significantly accelerated the progress of pharmacognosy at the end of the 20th century. Relevant disciplines and knowledge were integrated into pharmacognosy, the connotation and denotation of pharmacognosy were expanded, the research content was enlarged, and new techniques and methods were developed. From these advances various difficulties have emerged as well. The following questions are typical examples. How might the qualitative variability observed in medicinal raw materials be defined? What is the material basis for this variability? How do high-quality medicinal materials (especially top-geoherbs) form, and what is the molecular basis and the environmental mechanism for such formation? [12] What is the biological mechanism of active-components accumulation in medicinal raw materials, and what are the factors influencing these mechanisms? How to increase the content of active components? What are the characteristics of the germplasm resources of medicinal raw materials, and what are the differences between the germplasm resources of medicinal raw materials and the plants as crops?

Recently, we began to realize that studies about germplasm resources and conservation of endangered species and regulation of secondary metabolites could not be restricted at the level of organisms, tissue, organs, or cells. Developments in pharmacognosy emphasize the urgency of explaining biological questions at the molecular level, including genes, proteins and enzymes. Studies concerning biological phenomena related to pharmacognosy rarely focus on molecular mechanisms. As a result, there is an urge to investigate pharmacognosy more at the molecular level in order to meet the requirements of research and development, e.g., expanding fundamental knowledge at the molecular level, and solving production problems.

1.5 Molecular pharmacognosy: The natural outcome of the convergence of pharmacognosy and molecular biology

Molecular pharmacognosy is a product of the intersection of pharmacognosy and molecular biology. Its formation and development have been benefited from contributions from many other sciences, including molecular genetics, molecular systematics, molecular ecology, conservation biology and medicinal plant breeding science. Molecular pharmacognosy investigates medicinal raw materials at the level of nucleic acids and proteins. Its emergence is the inevitable outcome of a trend to make pharmacognosy incorporate sub-cellular factors.

2 Molecular pharmacognosy and pharmacognosy and the significance of the formation of molecular pharmacognosy

2.1 Molecular pharmacognosy and pharmacognosy

Molecular pharmacognosy has a comparable outlook to conventional pharmacognosy, but also entails new objectives and challenges. Xie ZongWan view is that molecular pharmacognosy and conventional pharmacognosy complement, rather than replace one another [10]. As they differ in their methods and techniques, these two disciplines investigate different problems with varying achievements. Therefore, a combination of all methods, or a selection of methods, from both pharmacognosy and molecular pharmacognosy may be preferable [10]. The systemic comparison between pharmacognosy and molecular pharmacognosy reported here aims to demonstrate that they promote, rather than substitute, one another (Table 1).

2.2 Significance of the formation of molecular pharmacognosy

The main objectives of research in pharmacognosy are to achieve the identification and quality assessment of individual plants and plant populations, or provide scientific bases for the production and sustainable use of medicinal raw materials resources. Such studies are conducted at the cellular, tissue, organism and population levels. Mature and independent theories and methods, such as organism pharmacognosy and morphological pharmacognosy, were developed at these levels. By contrast with the mission of pharmacognosy, molecular pharmacognosy aims to identify particular molecules in medicinal raw materials, study the background of their origin, reveal the molecular mechanisms of secondary metabolite accumulation, understand the principles governing the biosynthesis of secondary metabolites, and provide scientific bases for high-quality production and conservation of medicinal raw materials.

The significance of the development of molecular pharmacognosy lies in two main aspects. Firstly, it expanded the study of pharmacognosy into the microscopic and genetic realms, and extensively enriched the understanding of biological phenomena in medicinal raw materials. Secondly, it facilitated the classification of cells, tissues, organs, organisms and populations of medicinal raw materials, as different genes and DNA segments have different evolution rates, have special positions in evolution, and reflect different scales and levels of genetic variance. Researchers recognized both the relevance and limitations of the study of

these phenomena. Therefore, they analyzed and integrated all studies at all levels in order to obtain a comprehensive understanding of medicinal raw materials. Their efforts extended the depth and scope of pharmacognosy, and transformed it from phenomenology to a modern and systematic subject. These developments led to special views at first, and subsequently to specific scientific questions and ideas. Methods and theories for the study of these questions arose. Finally, molecular pharmacognosy emerged. Xie ZongWan, a highly-reputed pioneer of pharmacognosy, identified that the initial publication of *Molecular Pharmacognosy* in 2000 was a milestone in the foundation of a new division of pharmacognosy [10].

3 The scope of research, characteristics and techniques of molecular pharmacognosy

3.1 Scope of research

3.1.1 Molecular identification of medicinal raw materials

The molecular identification of medicinal raw materials is the primary objective of molecular pharmacognosy. A DNA marker is a detectable genetic marker characterized by good veracity and reproducibility. Molecular identification has the advantage of not being impacted by environmental factors and changes in appearance and description that result from the processing of the studied medicinal materials. This is in sharp contrast with traditional identification methods, which are based on the origin, characteristics, microscopy and physico-chemistry of the material. Drawing on these methods, molecular identification allows the morphological variations of medicinal raw materials to be described much more precisely than descriptions made at the morphological, tissue, or chemical level. Many molecular identification techniques are commonly used. They are classified according to their technological bases. Random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), arbitrarily primed polymerase chain reaction (AP-PCR), MARMS, APAPD and PCR-RFLP are based on a combination of polymerase chain reaction (PCR) and electrophoresis (EP) [18]. Single nucleotide polymorphisms (SNP) and DNA barcoding are based on DNA sequencing. More than one hundred academic papers concerning the molecular identification of medicinal raw materials are available in the Academic Literature Full-text Database of China, in relation to *Trichosanthes kirilowii* (Tianhuafen), *Panax ginseng* (Renshen), *Angelica sinensis* (Danggui), *Angelica sinensis* (Dahuang) and *Bupleurum chinense* (Chaihu) [19,20].

3.1.2 Phylogenetic evolution of medicinal plants and animals

In addition to serving as the basis for classification, the elucidation of the phylogenetic trees of medicinal plants and animals is also the basis for the identification of alternative materials or new drugs among species which are closely related to known medicinal species. In contrast to traditional identification methods which focus on phenotype, molecular biological identification is scarcely influenced by envi-

Table 1 A comparison between pharmacognosy and molecular pharmacognosy

	Pharmacognosy	Molecular pharmacognosy
Concepts	Pharmacognosy is the science of the origin, identification, active components, production, collection, quality evaluation and sustainable use of resources of medicinal raw materials [14].	Molecular pharmacognosy is the science of the classification, identification, cultivation and conservation of medicinal raw materials, as well as the production of their active components at a molecular level [15].
Object	Pharmacognosy, while focusing on applications, essentially determines identity and quality at the level of individuals, populations, or other macroscopic levels, for the purpose of providing scientific bases for the production and sustainable use of medicinal raw materials resources.	Molecular pharmacognosy, while focusing on mechanisms and applications, identifies molecules in medicinal raw materials, studies their origins, reveals the molecular mechanism of secondary metabolite accumulation, investigates the principles of molecular modulation and the biosynthesis of secondary metabolites, and provides scientific bases at a molecular level for the high-quality production and conservation of medicinal raw materials.
Core of research	Cognizance and identification of the origins of medicinal raw materials, investigations and documentation searches of resources of medicinal raw materials, establishment of quality-control standards and quality evaluation of medicinal raw materials, GAP for TCM materials, resource development study [14,16].	Phylogenetic evolution of medicinal plants and animals, germplasm resource evaluation, and the conservation of medicinal plants and animals, mechanisms of endangerment, and the conservation of medicinal plants and animals, biosynthesis and regulation of active components in medicinal plants, characteristics of top-geoherbs, and molecular mechanisms in medicinal plants and animals, molecular identification of medicinal raw materials [17].
Primary investigative methods	Origin identification, character identification, microscopic identification, physicochemical identification and chemical ingredient identification.	DNA analysis technology (which consists of molecular hybridization), molecular markers, gene chip and gene engineering techniques; protein analysis technology, biological transformation technology, and the traditional analytical methods of pharmacognosy, including origin identification, microscopic identification, physicochemical identification and chemical ingredient Identification.
Closely-related fields	Herbology, TCM resource, TCM identification, and TCM chemistry and analytical chemistry.	Pharmacognosy, molecular biology, molecular genetics, molecular ecology, plant physiology, biology and genetics.

ronment variables. It therefore better reflects the essence of organisms in their evolution, and thereby yields more reliable research data. The crux of basic research in molecular pharmacognosy is the description of differences in the genetic backgrounds and relationships between medicinal plants and animals, by means of DNA molecular genetic markers, genetic sequencing, protein analysis, chromosome counts and other molecular techniques at the level of populations, individuals and genes. Another critical factor is the determination of phylogenetic trees for significant medicinal plants and animals on the basis of the gene sequence analyses of chloroplast and nuclear genomes, and the confirmation of genetic relationships and the relative positions of these plants and animals. Genes such as *rbcL*, *matK*, *rps4*, 18s rRNA and ITS are commonly used for molecular systematic analysis. The first three genes are part of the chloroplast genome, and the last two belong to the nuclear genome. Many relevant reports have been published, including studies about *Angelica* (Baizhi) [21], *Trichosanthes kirilowii* (Gualou) [22], *Codonopsis* (Dangshen) [23], *Atractylodes lancea* (Cangzhu) [24], *Paeonia*(Shaoyao) [25], *Magnolia officinalis* (Houpo) [26], and *Zaocys dhumnades* (Wushaoshe) [27].

3.1.3 Evaluation and preservation of germplasm resources

Germplasm resources, also called genetic resources, include various propagative materials for cultivation and wild propagation, as well as genetic materials produced artificially from those propagative materials [28]. Germplasm resources are fundamental materials used in the selection and propagation of new medicinal species, and are major factors in the quality and yield of TCM. In addition, they are key factors in the selection and propagation of medicinal species, and in the sustainable use of available resources. The collection, grouping, conservation and evaluation of germplasm resources are essential elements of the study of germplasm resources of medicinal plants and animals. Several years ago, when scientists selected high quality species for propagation, the phenotype diversity of many germplasm resources, such as the phenotype diversity of *Taxus chinensis* (Hongdoushan), *Panax ginseng* (Renshen), *Lycium barbarum* (Gouqizi) and *Rehmannia glutinosa* (Dihuang), became apparent. Molecular techniques were later introduced into the field of germplasm resource studies. The diversity and purity of germplasm resources, genetic structure, and the relationship between phenotype and genetic characteristics, were observed in *Dendrobium loddigesii* (Shihu) [29], *Magnolia officinalis* (Houpo) [30], *Dendranthema morifolium* (Juhua) [31], *Paeonia lactiflora* (Shaoyao) [32], *Scutellaria baicalensis* (Huangqin) [33], *Angelicae dahuricae* (Baizhi) and *Atractylodes lancea* (Cangzhu). Those studies afforded genetic bases for germplasm resource conservation.

3.1.4 Mechanism of endangerment and protection of endangered medicinal plants and animals

Genetic diversity is critical for populations of medicinal plants and animals to be able to adapt to environmental changes, and is thus essential for long-term survival. Without genetic diversity, plants and animals cannot compete in general evolution. Various internal and external factors result in the endangerment of medicinal plants and animals. A lack of genetic diversity is one of the key internal factors. Therefore, conservation of genetic diversity has become an essential objective in the protection of endangered species. Molecular markers based on DNA polymorphisms and gene sequences based on molecular systematics facilitate the evaluation of DNA variation, thereby identifying which plants and animals should be more actively protected. Because the process of the evolution of sub-species populations is fundamentally similar to that of the phylogenetic evolution of entire species, it is possible to predict, on the basis of systematic molecular studies of medicinal plants and animals, the prospective growth and possible endangerment of populations. In addition, innovative, practicable, tools may be designed for the measurement of biodiversity and the conservation of rare medicinal plants and animals such as *Cistanche deserticola* (Roucongrong) [34], *Eucommia ulmoides* (Duzhong) and *Panax notoginseng* (Sanqi). Significant advances achieved through population genetic variation studies, particularly by means of molecular phylogeographical techniques, have resulted in new theories and methods for the study of genetic variation within populations of medicinal raw materials [35,36].

3.1.5 Biosynthesis and regulation of active components in medicinal plants

In raw materials, components used for their medicinal activity mostly consist of secondary metabolites. Hence, the quality of a medicinal raw material primarily depends on the presence and amounts of secondary metabolites. Studies concerning the formation, regulation and biosynthesis of secondary metabolites, as well as genetic engineering to increase the levels of active components in raw materials, have become the focus of research in molecular pharmacognosy. For example, two new components showing higher activities than their precursors were obtained by the biotransformation of dl-tetrahydropalmatine, an analgesic molecule from *Corydalis yanhusuo* (Yanhusuo). Coumarins have been transformed by means of transgene techniques in the hair root culture of *Polygonum multiflorum* (Heshouwu) and the crown gall culture of *Panax quinquefolium* (Xiyangshen). A majority of these new compounds are advanced glycation-end products (AGEs), and several of them have higher activities than their precursors. Triptolide from *Tripterygium wilfordii* (Leigongteng) has been transformed by *Cunninghamella echinulata* AS3. 970, and four new

chemical compounds with cytotoxic activity on human tumor cell lines have been obtained through this biotransformation [37]. Dozens of hair root culture systems have been developed for medicinal plants, including *Lithospermum erythrorhizon* (Zicao) [38], *Catharanthus roseus* (Changchunhua) [39], *Panax ginseng* (Renshen) [40], *Salvia miltiorrhiza* (Danshen) [41], *Artemisia annua* (Qinghao) [42] and *Glycyrrhiza uralensis* (Gancao) [43]. The genetic regulation of active components has been studied for dozens of medicinal plants, including *Papaver somniferum* (Yingsu), *Artemisia annua* (Qinghao) [44], *Salvia miltiorrhiza* (Danshen) [45], *Taxus chinensis* (Hongdoushan) [46] and *Camptotheca acuminata* (Xishu) [47]. We used *Agrobacterium tumefaciens* to infect *Asparagus officinalis* (Shidiaobai), which resulted in the production of crown galls with a high percentage of auindine alkaloids [48]. *Bidens sp.* (Guizhencao) was used to obtain many polyacetylene compounds [49], including *Catharanthus roseus* (Changchunhua) to produce abundant alkaloids [50], and *Digitalis purpurea* (Maodihuang) to produce cardiotonic steroids [51]. Transgenic techniques have been applied for the enhancement of stress tolerance in medicinal plants, the monitoring and forecast of disease and insect infestation in plants, as well as mitigation of drought and excessive salinity. Some transgenic techniques also increased the percentage of active components in the hair roots of such medicinal plants as *Salvia miltiorrhiza* (Danshen) and *Hyoscyamus niger* (Tianxianzi) [52] .

3.1.6 Characteristics of top-geoherbs and their molecular bases

"Top-geoherb" is an English approximation of an ancient term referring to TCM produced in specific areas with superior qualities. Because special genotypes are extensively regulated in their local biotope, and key metabolic enzymes are expressed in different ways, the formation of top-geoherbs has been shown to be a special process [53,54]. The study of top-geoherb characteristics is a special field within molecular pharmacognosy. Studies of their molecular mechanisms have the following objectives: exploration of genetic variation in populations of top-geoherbs at the molecular level, clarification of genotype characteristics, determination of environmental influences on gene expression, and the study of the contribution of genetic factors to top-geo- herb formation. It has been reported that the genetic essence of top-geoherbs at the population level is most often the object of quantitative changes. This is because the main differences between top-geoherbs and other herbs from the same species are felt to be primarily due to changes in genotypic frequency at the population level and to quantitative inheritances under minor-polygene regulation, or quantitative genetics under the combination of key-gene and minor-polygene regulation at the individual level. Some specific achievements have provided a basis for studying top-geoherb formation mechanisms at the genetic, environmental and signal transduction levels including: cloning of gene coding for the syntheses of secondary metabolites in medicinal plants [55,56], studies of relevant transcription regulating factors [57] and intracellular signal transduction of external stimuli [58]. Several materials have been extensively studied for their molecular mechanisms, i.e. *Paeonia lactiflora* (Shaoyao), *Atractylodes lancea* (Cangzhu) [24], *Pogostemon cablin* (Guanghuoxiang), *Magnolia officinalis* (Houpo) and *Gardenia jasminoides* (Zhizi).

3.2 Characteristics of the subject

The characteristics of molecular pharmacognosy, while revealing the complexity and difficulty of research are also the impetus driving its formation and development. Those characteristics include the following three aspects.

3.2.1 The research field is broad and markedly interdisciplinary

Pharmacognosy is inherently an applied and interdisciplinary subject, whereas molecular pharmacognosy is being developed on the bases of pharmacognosy and other cognate fields as a comprehensive borderline subject. Concepts and techniques from pharmacognosy, molecular biology, molecular genetics, molecular ecology, plant physiology, genetics, chemistry of TCM, analytical chemistry and biological chemistry, are applied to research in molecular pharmacognosy. This fact emphasizes its strongly interdisciplinary character. The development of molecular pharmacognosy critically rests on the expertise of scholars who integrate various sciences into this field [59]. The training of multi-skilled personnel and the building of an expert team is a long-term project and speaks to the inter-disciplinary nature of the field.

3.2.2 Studies about the accumulation of secondary metabolites are a feature of every section of molecular pharmacognosy

The chief difference between medicinal and non-medicinal plants and animals is in their medicinal value. That value is the standard for the quality appraisal of medicinal raw materials. Whatever the shape, properties, odor, taste or other external characteristics, it is the class, accumulation and proportions of secondary metabolites which constitutes the quality standard of medicinal materials. A wide view of molecular pharmacognosy indicates that many relevant studies in this field are directly or indirectly related to the accumulation of secondary metabolites. These study topics include the biosynthesis and regulation of active components in medicinal plants, the molecular distinction between genuine and counterfeit materials and between high-quality and lower-quality ones, the characteristics of top-geoherbs with a focus on their superior quality as medicinal materials, the quantitative evaluation of germplasm resources, the

mechanisms of endangerment, and the conservation of medicinal plants and animals. Studies about the accumulation of secondary metabolites are a prominent feature of every facet of molecular pharmacognosy. Investigation and regulation of the formation and accumulation of secondary metabolites at the molecular level are the major objectives of research in molecular pharmacognosy. In this respect, molecular pharmacognosy significantly differs from the study of non-medicinal plants, e.g. crops or forest species, and animals [15].

3.2.3 Research topics are rich, but suffer from the difficulty of defining model plants for study

Molecular pharmacognosy studies cover more than ten thousand medicinal plant and animal species, hundreds of which are commonly used. In comparison with crops and forest species, which include a few dozen of the commonly studied subjects, molecular pharmacognosy has an abundance of subjects for investigation. In addition, the mechanisms of secondary metabolite formation, as a primary feature of molecular pharmacognosy, are complex and diverse. There are as many as five core biosynthesis pathways with general cognizance. The fact that those pathways are interwoven with complicated metabolic networks underlines the difficulty of the search for an ideal model plant for the investigation of secondary metabolites, the core of molecular pharmacognosy.

3.3 Techniques

The main techniques used in molecular pharmacognosy include the following: DNA analysis (consisting of molecular hybridization), molecular markers, gene chip and gene engineering, protein analysis (particularly enzyme technology), and biological transformation. The typical analytical methods used in pharmacognosy including origin identification, microscopic identification, physicochemical identification and chemical ingredient identification [1,4,60]. A few of these techniques are introduced in the following sections.

3.3.1 DNA analysis technology

DNA analysis technology mainly consists of molecular hybridization, molecular markers, gene chip and gene engineering.

(1) Molecular hybridization. Molecular hybridization is a molecular technique which determines the base sequence of single-stranded RNA. It is usually used to identify RNA bases and estimate the genetic similarity of target genes. It primarily consists of solid-phase hybridization and solution hybridization, and includes colony *in situ* hybridization, dot blotting, Southern blotting, northern blotting and tissue *in situ* hybridization.

(2) DNA markers. A DNA marker is a type of genetic marker based on polymorphic DNA. It facilitates the differentiation of genetically distinct individuals through the direct analysis of polymorphic DNA. It is used for the identification of medicinal raw materials and the exploitation of new medicinal sources.

DNA markers are classified in the following categories: (i) Molecular markers based on molecular hybridization. For example: restriction-fragment length polymorphism (RFLP), variable number of tandem repeats (VNTRs) and denaturing-gradient gel electrophoresis-RFLP (DGGE-RFLP). (ii) Molecular markers based on PCR. For example: randomly amplified polymorphic DNA (RAPD), arbitrary primer-PCR (AP-PCR), DNA amplification fingerprinting (DAF), single-strand conformation polymorphism-RFLP (SSCP), sequence-characterized amplified region (SCAR), cleaved amplified polymorphism sequences, also called PCR-RFLP (CAPS), amplified fragment length polymorphism (AFLP), allele-specific PCR (AS-PCR), single primer amplification reaction (SPAR), simple sequence repeat, also called micro satellite DNA, or short tandem repeat (SSR), and inter simple sequence repeat (ISSR). (iii) Molecular markers based on the integration of PCR and RFLP. For example: amplified fragment length polymorphism (AFLP). (iv) Molecular markers based on a reverse-transcription polymerase chain reaction. For example: reverse transcription PCR (RT-PCR), differential display (DD), representative difference analysis (RDA), fluorescence quantitative PCR (FQ-PCR). (v) Molecular markers based on sequencing. For example: single nucleotide polymorphisms (SNP), DNA barcoding. (vi) DNA chips. For example: cDNA microarray and oligo microarray.

(3) Gene chips. The gene chip, or DNA microarray technique, probes molecules that are fixed on DNA microarrays at high densities, usually over 400 per cm^2, to be hybridized to the target molecules. Doing so determines the expression level of the hybrid signal of every probe molecule for each site on the array, thereby obtaining information about the target molecules and sequencing. As a new molecular hybridization and sequencing method, the DNA microarray technique facilitates the determination and assay of a large number of target molecules. Thus, it is not handicapped by disadvantages including the complicated operation procedures involved in conventional blotting hybridization techniques, low levels of automation, limited sequencing, and low efficiency. This technique is used for the determination of gene expression profiles, analysis of polymorphisms, construction of a genomic library, analysis of mapping, and sequencing by hybridization. The gene chip technique has been employed to study environmental influences on gene expression in top-geoherbs, and to analyze the genetic modulation of their primary active components [61].

(4) Recombinant DNA technique. The recombinant DNA technique, also called gene cloning, or molecular cloning, is the basis for molecular engineering. It includes a series of laboratory techniques whose final objective is the introduction of DNA from one organism into another. Drawing upon the discovery of homologous recombination of DNA, mo-

lecular engineering has the following advantages: it needs no restriction endonuclease and DNA ligase, no change of normal DNA recombination protocols. Numerous cloning vectors are available. This technique has primarily been used for genetic modifications in medicinal raw materials, and to develop vectors for the biosynthesis of secondary metabolites.

3.3.2 Protein analysis

Protein analysis is mainly used to analyze variations in medicinal raw materials at the protein level. It focuses on protein isolation and purification, the identification of proteins, the salting-out and dialysis of proteins, the electrophoresis of proteins, and staining methods for proteins. Protein electrophoresis techniques include cellulose acetate membrane electrophoresis, agarose gel electrophoresis, polyacrylamide gel electrophoresis (PAGE), SDS-PAGE, electrophoretic blot transfer, isoelectric focusing (IEF), two-dimensional polyacrylamide gel electrophoresis, and immunoelectrophoresis.

3.3.3 Biotransformation

This technique is primarily used in studies about the biosynthesis and accumulation of secondary metabolites. In this field, research is mainly related to the creation and selection of biotransformation systems (including microorganisms, cells in suspension cultures, and transgenic organs), the formation and cultivation of crown galls and hair roots, the expression induction of active components through addition of extrinsic substrates [62], as well as their extraction, isolation and identification of biotransformation [63].

4 Outlooks for further developments in molecular pharmacognosy

4.1 Sustainable uses of medicinal raw material resources and advances in relevant techniques

The combination of requirements aiming at the sustainable use of raw material sources and advances in relevant techniques is significant for molecular pharmacognosy research. Future molecular pharmacognosy research will likely focus on the phylogenetic evolution of medicinal plants and animals, the mechanisms of endangerment, the protection of endangered medicinal plants and animals, and the top-geoherbs.

4.1.1 Molecular identification and DNA barcoding

The Chinese Academic Literature Full-text Database documents and collections related to molecular pharmacognosy have measurably expanded. Concepts and techniques have been widely adopted, as the cost of apparatus and reagents declined. However, more rapid methods for molecular identification are still required. Because of its function in species identification, DNA barcoding may become a standard identification method for medicinal raw materials at the molecular level.

4.1.2 Functional genome research related to secondary metabolites

Genomics comprises structural genomics, which focuses on sequencing the complete genome, and functional genomics (also designated as post-genomics), which aims at the identification of gene functions. Genomics has evolved from the study of static RNA-base sequences to that of the dynamic biological functions of the genome. Studies about molecular mechanisms and the regulation of secondary metabolism are characteristics of molecular pharmacognosy. One of their major objectives is to enable the acceleration and modulation of the biosynthesis of secondary metabolites. Fundamental research into secondary metabolic pathways has investigated gene coding for the key enzymes involved. Research in genomics focusing on the functions of secondary metabolites is vital for molecular pharmacognosy [64], as are advances in genetic engineering, tissue culture and biotransformation.

4.1.3 The integration of proteomics, genomics and metabolomics

Genomics, proteomics and metabolomics are jointly used in molecular pharmacognosy at the molecular level, with each possessing specific advantages and objectives. Genomics primarily focuses on functional genes at the genetic level. Proteomics chiefly concerns protein expression at the protein level. Metabolomics essentially focuses on secondary metabolites. Secondary metabolites have a particular multigene character, and their accumulation is strongly impacted by the environment, especially environmental stress. As a result, variations are usually induced at the level of gene expression and proteins. The integration of genomics, proteomics and metabolomics will directly benefit molecular pharmacognosy [65].

4.1.4 New ideas and methods for the core collection

The core collection is one of the key subclasses of germplasm resources. The collection has been designed to preserve maximal genetic diversity and to represent the geographic distribution of entire populations with minimal genetic resources. Studies of the core collection will improve the management and the utilization of the germplasm database. The core collection of medicinal raw materials is primarily gathered as a core collection of crops. From existent germplasm databases or large germplasm resources for crops, 10% of samples have been drawn according to scientific methods and techniques. Those samples may represent, to a certain extent, the spectrum of morphologies, geographic distributions, genes and genotypes of a species and its wild related species. However, there exist clear differences between the germplasm resources of crops and those

of many medicinal raw materials.

Many kinds of medicinal raw materials do not have their own germplasm databases. Sometimes, a large number of wild species hamper the creation of germplasm databases, especially for rare and endangered species, whose germplasm cannot be gathered in large quantities. This problem may be related to inadequate research bases for medicinal raw materials, or to the great variety and limited germplasm of most medicinal species. It is clear that the construction of germplasm databases for medicinal raw materials cannot copy the model used for crops databases.

The techniques and methods of molecular pharmacognosy for the determination of genetic diversity in medicinal raw materials should be maximized within the context of limited germplasm resources. Those efforts should include the analysis of differences in the genotypes of medicinal plants and animals, as well as the distinct responses of various genotypes to their environments. Based on the studies of genetic structures and the statistics of mixed linear models, best linear unbiased prediction (BLUP) will be implemented to predict a genotype for estimating genetic distances and appraisal of the genetic similarity of different materials. Subsequently, the germplasm database should be further developed by rational sample selection. To summarize, it is critical to design unique models for the core collection of germplasm resources of medicinal raw materials.

4.2 The theoretical background of molecular pharmacognosy

Six years elapsed from the first introduction of the concept of molecular pharmacognosy to the publication of the first edition of *Molecular Pharmacognosy*. Eight years later, this publication was listed among other innovative teaching materials for higher education in China. Biodiversity and intra-species research raised issues concerning the standardization of medicinal raw materials and related strategies. Research about minor genes linked to secondary metabolites, the interaction between genes and the environment, and the understanding of the gene network and sequenced expression, are fundamental for study of gene regulation and selection of medicinal species [12]. The complicated nature of the biosynthetic pathways of secondary metabolites requires systemic engineering with unique theories, methods and techniques. These fundamental developments emphasize the significance of biodiversity in molecular pharmacognosy and underscore the complexities arising from pharmacognosy studies at the molecular level.

4.3 Applications of molecular pharmacognosy

Pharmacognosy is a subject emerging from practice. Although the study of molecular mechanisms enriches the theory of molecular pharmacognosy, applications remain those of conventional pharmacognosy. Practical questions arising in the production and utilization of medicinal raw materials remain the focal point of molecular pharmacognosy. Practical achievements are crucial to the existence of this subject, and also serve as hallmarks against which we can measure the healthy development of this discipline. To date, study fruits from the systemic catalogue of *Trichosanthes L*. have been adopted by the *Flora of China* (Vol. 19) and the *Pharmacopoeia of China*. Molecular pharmacognosy is an area of research which will undoubtedly undergo intensive development in the near future.

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