CrossMark

Review

Life & Medical Sciences

Recent advances in biosynthesis of bioactive compounds in traditional Chinese medicinal plants

**Lei Yang · Changqing Yang · Chenyi Li ·
Qing Zhao · Ling Liu · Xin Fang · Xiao-Ya Chen**

Abstract Plants synthesize and accumulate large amount of specialized (or secondary) metabolites also known as natural products, which provide a rich source for modern pharmacy. In China, plants have been used in traditional medicine for thousands of years. Recent development of molecular biology, genomics and functional genomics as well as high-throughput analytical chemical technologies has greatly promoted the research on medicinal plants. In this article, we review recent advances in the elucidation of biosynthesis of specialized metabolites in medicinal plants, including phenylpropanoids, terpenoids and alkaloids. These natural products may share a common upstream pathway to form a limited numbers of common precursors, but are characteristic in distinct modifications leading to highly variable structures. Although this review is focused on traditional Chinese medicine, other plants with a great medicinal interest or potential are also discussed. Understanding of their biosynthesis processes is critical for producing these highly value molecules at large scale and low

L. Yang · L. Liu · X.-Y. Chen (✉)
Plant Science Research Center, Shanghai Chenshan Botanical Garden, Shanghai Key Laboratory of Plant Functional Genomics and Resources, Shanghai 201602, China
e-mail: xychen@sibs.ac.cn

C. Yang · C. Li · Q. Zhao · X. Fang · X.-Y. Chen
National Key Laboratory of Plant Molecular Genetics and National Center for Plant Gene Research, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

C. Li
University of Chinese Academy of Sciences, Beijing 100049, China

cost in microbes and will benefit to not only human health but also plant resource conservation.

1 Introduction

China is rich in plant resources. Of the ∼300,000 species of higher plants on the earth, around 10 % can be found in China. As in many other countries, people in China have used plants for treatment of diseases for thousands of years. *Compendium of Materia Medica* has been held in high esteem since it was first published in 1593, and this ancient encyclopedia of traditional Chinese medicine (TCM) described more than 1,000 species of plants. Plants produce a wealth of specialized (or secondary) metabolites also known as natural products, which are small molecular weight compounds with enormous structural diversity and show various biological activities. It is estimated that there are approximately 200,000 secondary metabolites in plant kingdom [1], which, based on biosynthetic origins, can be classified into three major categories: phenylpropanoids, terpenoids and alkaloids, plus a few other less abundant groups. The usage records of China's ancient medical books, such as *Sheng Nong's Herbal Classic*, *Huang Di's Canon of Medicine* and *Compendium of Materia Medica*, already recognized that plant extracts contain active principles in treating illness and classified them into assumptive, intuitive or largely philosophic categories, such as cold, neutral or hot, toxic or nourishing. Over the past century, hunting the active ingredients has led to important findings, such as artemisinin for malaria, huperzine A for Alzheimer's disease, ephedrine for cold and camptothecin

SCIENCE CHINA PRESS

🖉 Springer

for cancer, which were isolated from *Artemisia annua*, *Huperzia serrata*, *Ephedra sinica*, *Camptotheca acuminate*, respectively [2]. Very recently, tetrandrine, an alkaloid isolated from the TCM plant *Stephania tetrandra* previously used for reducing blood pressure, were reported to have the therapeutic efficacy against Ebola [3], and celastrol, a triterpene extracted from *Tripterygium Wilfordi*, has the potential as an anti-obesity agent [4]. These findings strongly support that TCMs are the reliable source for new therapies in treatment of lethally epidemic disease and long unsolved disease.

However, multi-classes of natural products are generated by each plant species. In addition, geographic distributions, growth conditions and harvesting seasons could significantly affect chemical compositions of the plant. Whereas one component may act as the active ingredient, the effects of a mixture of many ingredients are often uncertain and this has caused increasing concerns [5]; thus, the traditional practice of herbology has to face the challenges from modern medicine and the manufactures' requirement.

While plant natural products continue to be a prime source for drug discovery and development, supply of these compounds is often curtailed due to limitation of natural resources and/or low contents in plant. The biotechnological platforms, such as metabolic engineering of effective plant and microbial production, are urgently needed to ensure that the supply of bioactive natural products is sustainable and environmentally friendly, rather than at the expense of resource exhaustion [6–9]. A prerequisite to these solutions is the understanding of the biosynthetic pathways of these specialized metabolites, in particular the cloning and identification of enzymes and the regulatory factors.

In the past two decades, the rapid development in genomics and high-throughput technologies of chemical analysis, in combination with molecular biology tools, has accelerated the research of medicinal plants. In this review, we summarize the recent advances in the elucidation of biosynthetic pathways of secondary metabolites in, not exclusively, TCM plants. Although alkaloids are probably the most important resource for drug discovery and biosynthesis of these amino acid-derived compounds has been investigated intensively, there are, surprisingly to some extent, relatively few studies of alkaloids from TCM plant; thus, this review is emphasized on phenylpropanoids and terpenoids. In addition to enzymes, transcription factors characterized from medicinal plants are also discussed.

2 Phenylpropanoids

Phenylpropanoids, commonly found in plants, are derived from the six-carbon aromatic phenyl group and the three-carbon propene tail [10], and form a large group of specialized metabolites including monolignols, lignans, flavonoids, phenolic acids and stilbenes [11]. They serve as basic components of a number of structural polymers, as well as floral pigments, scent compounds or signaling molecules to mediate bio-interactions, phytoalexins against herbivores and pathogens, and protective components against ultraviolet light radiation and other abiotic stresses [12]. In many TCM plants, such as the plants of Lamiaceae, Fabaceae (Leguminasae) and Asteraceae, phenylpropanoids are also the bioactive principles (Table 1), which have been shown to act as anti-oxidants, free radical scavengers, anti-inflammatories and anticancer compounds [13].

The majority of phenylpropanoids are derived from phenylalanine. The first three steps are catalyzed by phenylalanine ammonia lyase (PAL), cinnamate

Table 1 List of examples of TCM plants rich in phenylpropanoids

Plant species	Chinese name in Pin-yin	Family	Representative compounds
Salvia miltiorrhiza	Danshen	Lamiaceae	Salvianolic acid A, B and C
Scutellaria baicalensis	Huangqin	Lamiaceae	Baicalin, wogonin, scutellarin
Glycyrrhiza uralensis	Gancao	Leguminosae	Liquiritin, isoliquiritin, 7,4′-dihydroxyflavone
Astragalus membranaceus	Huangqi	Leguminosae	Calycosin-7-glucoside, ononin
Sophora flavescens	Kushen	Leguminosae	Sophoraflavecromane A, B, C
Sophora tonkinensis	Shandougen	Leguminosae	Sophoranone, sophoradin
Pueraria lobata	Ge	Leguminosae	Puerarin, daidzin, genistein
Lonicera japonica	Jinyinhua	Caprifoliaceae	Chlorogenic acid, luteolin
Dendranthema morifolium	Juhua	Asteraceae	Chlorogenic acid, acacetin-7-*O*-β-D-glucoside, apigenin-7-*O*-β-D-glucoside, and luteolin-7-*O*-β-D-glucoside
Ginkgo biloba	Yinxing	Ginkgoaceae	Ginkgetin, isoginkgetin
Epimedium brevicornu	Yinyanghuo	Berberidaceae	Icariine, icarisid
Isatis indigotica	Songlan	Brassicaceae	Lariciresinol

4-hydroxylase (C4H) and *p*-coumaroyl coenzyme A ligase (4CL), which are commonly referred as "general phenyl-propanoid pathway" [14, 15]. The product of 4CL is used as precursor for the biosynthesis of various phenyl-propanoids in plants (Fig. 1). Parts of phenylpropanoids are synthesized from L-tyrosine, and the transformation is more

restricted, being mainly limited to members of several families. For instance, 3,4-dihydroxyphenyllactic acid, one precursor of rosmarinic acid, is synthesized from tyrosine-derived pathway in some species of Lamiaceae, such as *Salvia miltiorrhiza* [16, 17].

2.1 Flavonoids

Flavonoids constitute a highly diverse class of secondary metabolites composed of more than 9,000 structures [18]. They are commonly found in land plants, including all vascular plants and some mosses [19]. Based on the agly-cone core, they are generally further grouped into fla-vanones, flavones, flavonols, isoflavonoids, anthocyanins and proanthocyanidins. All flavonoids are basically derivatives of 1,3-diphenylpropan-1-one (C6–C3–C6), which is derived from the condensation of three malonyl-CoA molecules with one *p*-coumaroyl-CoA to form a chalcone intermediate [20]. Chalcone isomerase converts chalcone into flavanones, and respective enzymes trans-form flavanones to various flavones, isoflavones, dihydor-flavonols, flavonols and anthoanidins. Every class of flavanones possesses the compounds with pharmaceutical activity and is widely used in folk medicines [21].

2.1.1 Flavanones and flavones

Two completely different flavone synthase (FNS) proteins have been found to catalyze the biosynthesis of flavones in plants. The first member of the FNS I type was identified from parsley (*Petroselinum crispum*) cell suspension cul-tures and classified as 2-oxoglutarate-dependent dioxyge-nase [22]. The FNS I cDNA was then cloned and functionally expressed in yeast [23], and it shares a high sequence identity to the flavanone 3-β-hydroxylase (FHT). Interestingly, characterized FNS I enzymes appear to be mainly in the family of Apiaceae [18, 24]. Molecular and phylogenetic analysis revealed a gene duplication of FHT, and a subsequent neofunctionalization occurred early in the development of the Apiaceae subfamilies [25]. Formation of most flavones in a wide range of plant species is cat-alyzed by FNS II, cytochrome P450 proteins of CYP93B subfamily. The FNS II activity was first demonstrated in extract of *Antirrhinum majus* flowers [26], and the cDNAs were then isolated from other plants, including *Perilla frutescens* (CYP93B6) [27] and *Gentiana triflora* [28].

Glycyrrhiza uralensis is one of the most popular TCM plants and also widely used in food flavoring. Although the sweeting agent of this plant is glycyrrhizin, a triterpenoid saponin [29], flavanones and flavones are also important components in its root, which include liquiritigenin, isoliquiritigenin and 7,4′-dihydroxyflavone [30]. A P450 enzyme from *Glycyrrhiza echinata*, CYP93B1, was
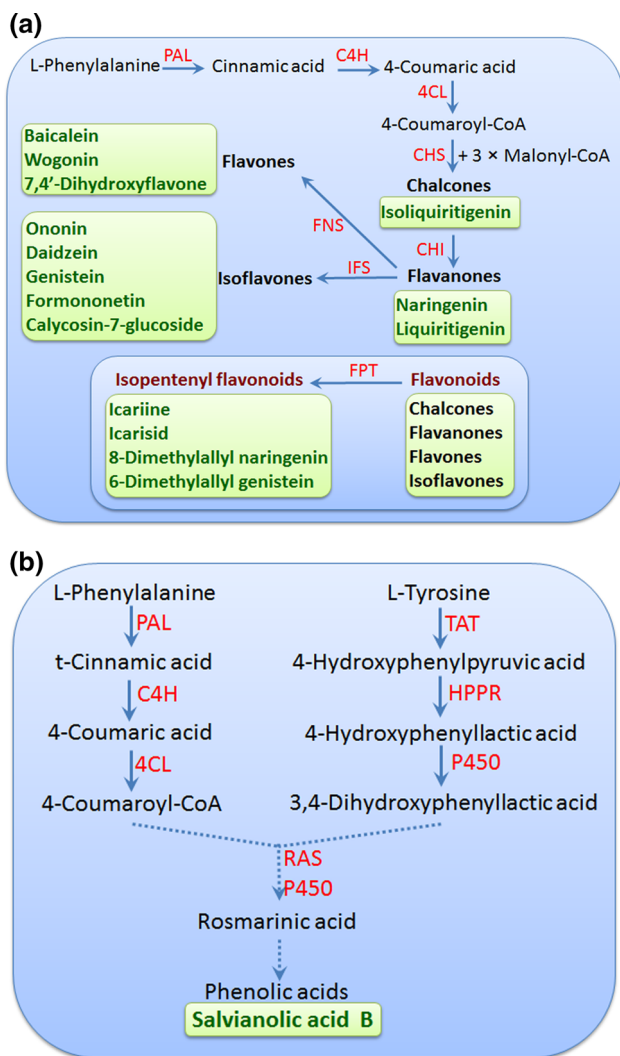

Fig. 1 (Color online) Biosynthesis of phenylpropanoids in TCM plants. **a** Biosynthesis of flavonoids and isopentenyl flavonoids; **b** formation of phenolic acids from the L-phenylalanine- and the L-tyrosine-derived pathways in *Salvia miltiorrhiza*, a medicinal plant of Lamiaceae. Phenylpropanoids are mainly synthesized from pheny-lalanine via the "general phenylpropanoid pathway", catalyzed by phenylalanine ammonialyase (PAL), cinnamate 4-hydroxylase (C4H) and p-coumaroyl coenzyme A ligase (4CL). The product of p-coumaroyl-CoA is used for the biosynthesis of flavonoids, isopen-tenyl flavonoids and phenolic acids. CHS, chalcone synthase; CHI, chalcone isomerase; FNS, flavone synthase; IFS, isoflavone synthase; FPT, flavonoid prenyltransferase; TAT, tyrosine aminotransferase; HPPR, 4-hydroxyphenylpyruvate reductase; RAS, rosmarinic acid synthase; P450, cytochrome P450 monooxygenase. Dotted lines represent multiple enzymatic catalyzed steps

identified as flavanone 2-hydroxylase (F2H), a member of FNS II [31]. The products, 2-hydroxyflavanones, were transformed into flavones in vitro in acid treatment, suggesting that an additional enzyme, probably a dehydratase, was involved in catalyzing the formation of flavones. A full-length cDNA of cytochrome P450 *CYP93C2* was isolated from the elicited *G. echinata* cells, which was shown to encode 2-hydroxyisoflavanone synthase [32].

The flavones baicalin and wogonoside, as well as their aglycones baicalein and wogonin, represent the dominant flavonoids in *Scutellaria baicalensis*, a perennial species of Lamiaceae and an important herb in Chinese traditional and clinical-orientated medicine. The flavones, such as baicalin and wogonin, are distinct for lacking a 4′-OH group but having a 6-OH group on their A-ring [33]. Genes encoding the upstream enzymes of the pathway, including PAL, C4H, 4CL, chalcone synthase (CHS) and chalcone isomerase (CHI), have been isolated [34, 35]. However, the enzymes committed to the formation of the *S. baicalensis*-type flavones remain unknown. It is also possible that specific enzyme isoforms are involved in the formation of cinnamoyl-CoA [36]. It has been reported that accumulation of these flavones was enhanced by jasmonate (JA) treatment, and a R2R3-MYB transcription factor, SbMYB8, was found involved in the regulation [37, 38].

2.1.2 Isoflavones

The isoflavones are well studied for their substantial health promoting benefits. They are found mainly in leguminous plants and are the major bioactive ingredients in soybean, *Astragalus*, *Pueraria lobata* [39]. Isoflavones are converted from flavanones by the isoflavone synthase (IFS). By using EST-based approach combined with enzymatic assays, P450s of CYP93C subfamily from soybean were shown to have such activities [40, 41]. Members of this subfamily with IFS activity were also reported in other leguminous plants, such as *Lotus japonicus* [42] and *Trifolium pratense* [43].

Astragalus membranaceus, a species of Fabaceae, has been used in TCM for thousands of years. *Astragaus* is considered an adaptogen because it is believed to help protect the body against stresses, including those of physical, mental or emotional [44, 45]. In China, *Astragalus* has been used to help patients with severe forms of heart disease in relieving symptoms, lowering cholesterol levels and improving heart function. Constituents of the *Astragalus* roots (radix astragali) include polysaccharides, triterpenoids (astragalosides) and isoflavones [46, 47]. Isoflavones such as calycosin-7-glucoside and ononin are considered the important active components in this medicine. Hairy root system of *Astragalus* was developed a long time ago to produce these ingredients [48, 49]. Research at molecular level in this plant is limited, but will help reveal

the biosynthetic pathway in this leguminous medicinal plant [50].

Pueraria lobata, also a species of Fabaceae, is commonly known as "kudzu". Puerariae radix, the dried root of the kudzu, has been used in China as herbal medicine for the prevention of cardiovascular disease and rehabilitation of stroke patients [51]. The major secondary metabolites accumulated in kudzu roots are isoflavones, including daidzein, genistein, formononetin and their glucosides Puerarin [52], among which the 8-*C*-glucoside of daidzein is considered the major active compound [53]. The co-occurrence of both *O*- and *C*-linked glycosides in root is of particular interests and worthy of further investigation. Using a functional genomics approach, He et al. identified enzymes associated with the isoflavone biosynthesis in kudzu roots, including 15 UDP-dependent glycosyltransferases (UGTs), among which one, GT04F14, exhibited the in vitro activity of glycosylation of a wide range of substrates, including coumarins, flavones, flavonols, and isoflavones. The isoflavones are converted region-specifically to their 7-*O*-glucosides, whereas *C*-glycosylation might take place at the 2,7,4′-trihydroxyisoflavanone precursor of daidzein, rather than directly on daidzein. Conceivably the intermediate 8-*C*-β-glucopyranosyl-2,7,4′-trihydroxyisoflavanone is converted to puerarin under in vivo conditions by the action of 2-hydroxyisoflavanone dehydratase (HID). A candidate gene encoding HID was identified from the EST library of kudzu root [54]. In addition, a partially purified preparation from kudzu root was shown to have the *C*-glucosyltransferase activity that converted isoliquiritigenin (2′,4′,4-trihydroxychalcone) and UDP-Glc to puerarin [55].

2.1.3 Isopentenyl flavonoids

Prenylation, the addition of prenyl groups, contributes to the diversification of flavonoids, and the occurrence of more than 1,000 prenylated flavonoids in plants has been recorded [56]. This prenylation represents the coupling process of the aromatic moiety from shikimate pathway and the prenyl (isoprenoid) chain from the isoprenoid pathways. Many prenylated flavonoids were identified as active components in medicinal plants and thus are of particular interests as lead compounds for drugs and functional food ingredients [57].

Species *Sophora*, family Fabaceae, are widely distributed in Asia. *Sophora flavescens* has a long history of use in China, and the root, known as Ku Shen, is a typical TCM. It is used to dispel heat, dry dampness and eliminate intestinal parasites. It is thus administered in formulas for the treatment of dysentery and jaundice (damp-heat syndromes), edema and dysuria (dampness syndromes), and eczema and pruritis (damp-heat-wind syndromes). The *S.*

flavescens prenyltransferase SfN8DT-1 is the first enzyme identified to be responsible for the prenylation of naringenin at the 8-position, with dimethylallyl diphosphate (DMAPP) as substrate [58]. Later, two new flavonoid prenyltransferases (FPTs) were isolated from *S. flavescens* at the molecular level: one is the isoflavone-specific prenyltransferase (SfG6DT) for the prenylation of the genistein at the 6-position and the other a chalcone-specific prenyltransferase designated as isoliquiritigenin dimethylallyltransferase (SfiLDT) [29].

Herba epimedii is prepared from the aerial parts of *Epimedium brevicornum* or *Epimedium sagittatum,* species of Berberidaceae. Herba epimedii contains various bioactive components and has been utilized extensively in China as the tonic and anti-rheumatic herb for thousands of years, and in the treatments of diseases such as impotence, frequency/urgency of urination, coronary heart disease, chronic bronchitis and neurasthenia [59, 60]. The isopentenyl flavonoids icariine and icarisid are the major active compounds [61]; however, their biosynthesis remains poorly understood [62]. Recently, Huang et al. isolated 12 structural genes and two putative transcription factors (TFs) in the flavonoid pathway. Transcriptional analysis revealed that two R2R3-MYB TFs (EsMYBA1 and EsMYBF1), together with a bHLH TF (EsGL3) and WD40 protein (EsTTG1), are probably involved in coordinated regulation of biosynthesis of the anthocyanins and the flavonol-derived bioactive components [63].

2.2 Phenolic acids

Salvia miltiorrhiza is a perennial herb in the mint family (Lamiaceae). Its dried root or rhizome is called Danshen in TCM and was recorded in first pharmaceutical monograph *Shennong's Classic of Materia Medica* (A.D. 102-200). *S. miltiorrhiza* has been cultivated throughout Eastern Asia and used to prevent and cure cardiovascular, cerebrovascular, hyperlipidemia and acute ischemic stroke diseases [64]. Both the hydrophilic and lipophilic components in *S. miltiorrhiza* are considered active ingredients. The hydrophilic compounds are mainly phenolic acids including rosmarinic acid, salvianolic acid B, lithospermic acid and dihydroxyphenyllactic acid or Danshensu, and they may also function as antioxidative, anti-bacterial and anti-viral reagents [65, 66].

The biosynthetic pathway for phenolic acids in *S. miltiorrhiza* is distinct and has attracted many interests. Labeling experiments using [ring-(13C)]-phenylalanine suggested two intermediates derived from the phenylalanine-derived general phenylpropanoid pathway and the tyrosine-derived pathway, respectively (Fig. 1): 4-coumaroyl-CoA and 3,4-dihydroxyphenyllactic acid (DHPL), which are coupled by a acyl-CoA-dependent acyltransferase BAHD family enzyme rosmarinic acid synthase (SmRAS) to form 4-coumaroyl-3′,4′-dihydroxyphenyllactic acid (4C-DHPL). The 3-hydroxyl group is introduced later in the pathway by a P450 monooxygenase (SmCYP98A14) to form rosmarinic acid (RA) [16]. This type of P450 was first reported in *Coleus blumei* (Lamiaceae), and it catalyzes the 3-hydroxylation of 4-coumaroyl-3′,4′-dihydroxyphenyllactate and the 3′-hydroxylation of caffeoyl-4′- hydroxyphenyllactate, in both cases forming rosmarinic acid [67]. Recent genome assembly to search the putative enzymes involved in biosynthesis of phenolics in *S. miltiorrhiza* revealed twenty-nine candidates, among which 15 were predicted in the phenylpropanoid pathway, seven in the tyrosine-derived pathway and six encoding putative hydroxycinnamoyltransferases [17].

3 Terpenoids

Terpenoids are formed from sequential assembly of five-carbon building blocks (C_5H_8) called isoprene units. Accordingly, single or assemblies of two, three and four units constitute hemiterpenes, monoterpenes, sesquiterpenes and diterpenes, respectively. After the formation of the basic carbon skeletons, subsequent modifications, such as oxidation, reduction, isomerization and conjugation, lead to enormous numbers of structures, which represent the most abundant class of plant specialized metabolites, with more than 36,000 individual compounds [68].

In plant cells, the common precursors of terpenoids, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are synthesized via two independent pathways: the cytosolic mevalonic acid (MVA) pathway that starts with the condensation of acetyl-CoA, and the plastid-localized methylerythritol phosphate (MEP) pathway that uses pyruvate and glyceraldehydes 3-phosphate as substrates (Fig. 2). The IPP and DMAPP are condensed into geranyl diphosphate (GPP, C_{10}), farnesyl diphosphate (FPP, C_{15}) and geranylgeranyl diphosphate (GGPP, C_{20}) by the respective prenyltransferases and then converted to terpenes by terpene synthases (TPSs), which catalyze the critical step that determines the structures of terpen skeletons [69].

Generally, the cytosolic MVA pathway provides the precursor of FPP for the biosynthesis of sesquiterpenes and triterpenes, whereas the plastid MEP pathway is responsible for the biosynthesis of GPP and GGPP for mono-, di-, and tetra-terpenes [70]. Although cross-talk between these two spatially separated IPP pathways is prevalent, particularly in a direction from plastid to cytosol, our understanding of the molecular mechanism behind remains primitive.

3.1 Sesquiterpenoids

Monoterpenoids (C_{10}) and sesquiterpenoids (C_{15}) are widely distributed in plants, and they are the common constituents of volatile compounds in flowers, fruits, stems and leaves, playing important roles in plant–environment interactions, many of them also possess great commercial value and some are used in pharmaceuticals.

3.1.1 Artemisinin

One of the most famous plant-sourced medicines is artemisinin, an endoperoxide sesquiterpene lactone isolated from *Artemisia annua* L., an annual herb of Asteraceae. Due to its effectiveness against drug-resistant cerebral malaria, it is the essential component of the combinational therapies recommended by the World Health Organization [8]. It has saved millions of lives globally, especially in developing countries. The 2011 Lasker DeBakey Clinical Research Award and the 2015 Nobel Prize in Physiology or Medicine honor the Chinese scientist Youyou Tu who made the important contribution to the discovery of artemisinin [71–73].

As a sesquiterpenoid, artemisinin is believed to be synthesized from the cytosolic MVA pathway. However, a recent report suggested that the MEP pathway may also contribute to its biosynthesis. GPP, which is synthesized in plastids, can be transported to cytoplasm, forming FPP with the addition of another IPP unit [74]. The FPP is converted to the artemisinin skeleton by amorpha-4,11-diene synthase (ADS), a sesquiterpene synthase [75], and then oxidated by the cytochrome P450 CYP71AV1. When expressed in *Saccharomyces cerevisiae*, CYP71AV1 catalyzed the continuous oxidation of amorpha-4,11-diene into artemisinic alcohol and artemisinic aldehyde [76], with significantly increased production of artemisinic acid and artemisinic aldehyde when co-expressed with a cytochrome b5 (CYB5) in yeast [8]. The artemisinic aldehyde Δ11(13) reductase (Dbr2), a double-bond reductase, catalyzes the formation of dihydroartemisinic aldehyde [77], which is further converted into dihydroartemisinic acid by aldehyde dehydrogenase 1 (ALDH1) [78]. Moreover, an additional alcohol dehydrogenase (ADH1) was also found to be involved in the oxidation of amorpha-4,11-diene to artemisinic acid, with specificity toward artemisinic alcohol in *A. annua* plants [8].

Several transcription factors have been shown to participate in the regulation of artemisinin biosynthesis [79]. Two jasmonate responsive AP2/ERF proteins, AaERF1 and AaERF2, were found to up-regulate the transcription of *ADS* and *CYP71AV1* genes, by binding to the CRTDREHVCBF2 (CBF2) and RAV1AAT (RAA) motifs present in their promoters [80]. A WRKY family transcription factor, AaWRKY1, was demonstrated to be capable of binding to the W-box in the *ADS* promoter and involved in the regulation of artemisinin biosynthesis [81].

A deep sequencing on the transcriptome of *A. annua* to identify genes and markers for fast-track breeding was performed, and a detailed genetic map with nine linkage groups was built. Replicated field trials resulted in a quantitative trait loci (QTL) map that accounts for a significant amount of the variation in key traits controlling artemisinin yield, and positive QTLs in parents of new high-yielding hybrids were enriched, which made it available to convert *A. annua* into a robust crop [82]. Ma et al. [83] recently reported an integrated approach combining metabolomics, transcriptomics and gene function analyses to characterize gene-to-terpene and terpene pathway scenarios in a self-pollinating variety of *A. annua*. Forty-seven genes that mapped to the terpenes biosynthesis pathway were identified by sequence mining, and such metabolites-transcriptome network associated with different tissues is fundamental to metabolic engineering to artemisinin.
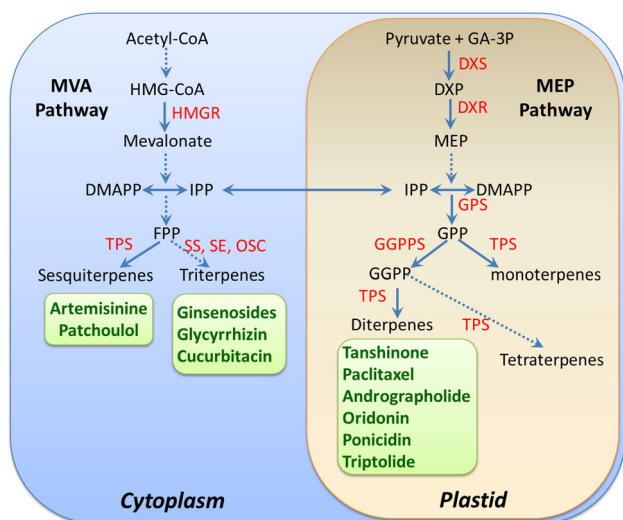

Fig. 2 (Color online) Biosynthesis of terpenoids in TCM plants. Terpenoids are synthesized via the cytosol MVA pathway and plastid MEP pathway. Generally, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) synthesized from the MVA pathway are converted to farnesyl diphosphate (FPP) for the biosynthesis of sesquiterpenoids and triterpenoids, whereas those derived from the MEP pathway contribute to the formation of geranyl diphosphate (GPP) and geranylgeranyl diphosphate (GGPP) for biosynthesis of monoterpenoids, diterpenoids and tetraterpenoids. HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MEP, 2-C-methyl-D-erythritol 4-phosphate; GGPP, geranylgeranyl diphosphate; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; GPPS, geranyl diphosphate synthase; GPP, geranyl diphosphate; FPPS, farnesyl diphosphate synthase; GGPPS, geranylgeranyl diphosphate synthase; TPS, terpene synthase; SS, squalene synthase; SE, squalone epoxidase; OSC, oxidosqualene cyclase. Dotted lines represent multiple enzymatic catalyzed steps

3.1.2 Patchoulol

Patchouli (*Pogostemon cablin*), a perennial herbaceous species of Lamiaceae, is not only a fragrant plant producing patchouli oil for cosmetics industry, but also a medicinal plant for the treatment of medical ailments, such as removing dampness, relieving summer heat and exterior syndrome, and serving as an anti-emetic and appetite stimulant [84]. The patchouli oil is composed of sesquiterpenoids dominated by (-)-patchoulol. The sesquiterpene synthase, patchoulol synthase, was firstly purified from patchouli leaves by chromatofocusing, anion exchange, gel permeation and hydroxylapatite chromatography [85]. Then, its cDNA was cloned and the recombinant patchoulol synthase was shown to produce patchoulol as the major product, plus at least 13 additional sesquiterpenes [86].

Patchouli oil in leaves accumulates with plant age: The content is low at juvenile stage and increases during plant growth and reaches a high level in mature plant. The microRNA156 (miR156)-targeted SQUAMOSA promoter binding protein-like (SPL) factors, which function as the major plant age cue in regulating developmental phase transition and flowering, play a key role in the age-dependent progressive up-regulation of the *patchoulol synthase* gene expression, and the patchouli oil biosynthesis. Interestingly, expression of a miR156-resistant form of SPL not only accelerated plant maturation but also promoted patchouli oil production [87].

3.2 Diterpenoids

Certain groups of diterpenoids (C_{20}), such as gibberellins, are regulators (phytohormones) of plant growth and development. Many other specialized diterpenoids, like tanshinone from *Salvia miltiorrhiza* and taxol from *Taxus*, are highly valuable in medicine. A few more examples include: stevioside, extracted from *Stevia rebaudiana* of Asteraceae, is a natural sweetest [88–90]; adenanthin, from the leaves of *Rabdosia adenantha*, induces differentiation of acute promyelocytic leukemia (APL) cells [91]; oridonin, from Lamiaceae plants *Isodon rubescens* and *Isodon amethystoides*, is a potential compound for molecular target-based therapy of leukemia [92]; and triptolide, a highly oxygenated diterpene isolated from *Tripterygium wilfordii*, was shown to have anti-leukemic activity [93].

3.2.1 Tanshinone

Besides the phenolic acids discussed above, tanshinones are another class of active diterpenoid compounds of *S. miltiorrhiza*, which include tanshinone I, tanshinone IIA, cryptotanshinone and dihydrotanshinone I. They are all abietane-type derivatives, among which tanshinone IIA is considered to be an important bioactive component in protecting cardiovascular system [94, 95], and tanshinone I was reported to be an apoptosis inducer and display anti-cancer activities [95].

As diterpenoid compounds, tanshinones are expected to be traced to the plastid MEP pathway, and their biosynthesis starts from the conversion of geranylgeranyl diphosphate (GGPP) to ent-copalyl diphosphate (CPP) and then to miltiradiene. The subsequent extensively structural tailing converts miltiradiene to cryptotanshinone, tanshinone I, tanshinone IIA or tanshinone IIB [96].

Based on sequence homology, enzymes shared by other diterpenoid biosynthesis have been characterized [96, 97]. To date, two enzymes specifically committed to the tanshinone biosynthetic pathway have been identified: the kaurene synthase-like (SmKSL), a diterpene synthase that utilizes CPP as substrate to produce miltiradiene [96], and a P450 monooxygenase CYP76AH1 which transforms miltiradiene to ferruginol [98], both representing the milestone achievement in the research of TCM plant. Recently, functional divergence of SmCPSs and SmKSLs was reported, which specified the roles of individual CPSs in tanshinone production in different tissues, including SmCPS1 in roots and SmCPS2 in aerial part, and SmCPS4 and SmKSL2 were found to oxidize ent-13-epi-manoyl in floral sepals, and the conserved SmCPS5 involved in the plant growth hormone gibberellin biosynthesis. This study is a typical example of how the evolutionary diversification of diterpenoids in plants in molecular level [99].

With the rapid development of sequencing technologies, several transcriptome datasets and the draft genome of *S. miltiorrhiza* have been reported. For examples, the cDNA library of whole plant contained 10,228 ESTs [100], the transcriptome of nearly entire growing cycle generated by Illumina revealed 56,774 unigenes [101], and the searching of the draft genome resulted in 40 putative genes encoding enzymes involved in the biosynthesis of universal isoprene precursors of IPP and DMAPP [102]. Genes encoding cytochrome P450 monooxygenases, dehydrogenases and reductases, as well as several groups of transcription factors were predicted to be involved in tanshinone biosynthesis by comparative analysis of transcriptomes generated from different tissues [103]. Recently, next-generation sequencing (NGS) and single-molecule real-time (SMRT) sequencing were combined to generate a more complete/full-length set of *S. miltiorrhiza* transcriptome, which provides a valuable resource for further investigation of tanshinone biosynthesis [104].

Organ- or tissue-specific patterns are common feature observed in biosynthesis and accumulation of specialized metabolites, as well as the expression patterns of corresponding genes [105–107]. Tanshinones are actively

synthesized and stored in roots, whereas only a low or trace amount was detected in aerial organs like leaves [108]. Moreover, both the accumulation and the expression of the related genes of tanshinones in hairy root cultures can be induced by biotic elicitors, such as the carbohydrate fraction of yeast extract, and phytohormones of salicylic acid and jasmonate [97, 109–114]. Further investigation can be directed to the characterization of the signaling components and transcription factors that regulate the diterpenoid biosynthesis in *S. miltiorrhiza*.

3.2.2 Taxol (paclitaxel)

Taxol (paclitaxel) is a diterpenoid isolated from the bark of *Taxus* trees. The anti-mitotic and cytotoxic properties of taxol are derived from its activity in disrupting normal tubulin dynamics, leading to dysfunction of microtubules [115]. Fourteen enzymes involved in taxol biosynthesis have been identified, they are geranylgeranyl diphosphate synthase [116], taxadiene synthase [117], taxadien-5α-ol-*O*-acetyl transferase [118], taxane 2α-*O*-benzoyltransferase [119], baccatin III: 3-anino-3phenylpropanoyltransferase [120], 10-deacetylbacctin III-10-*O*-acetyltransferase [121], 3′-*N*-debenzoyl-2′-deoxytaxol *N*-benzoyltransferase [122], taxane 5-alpha hydroxylase [123], taxane 10-alpha hydroxylase [124], taxane 13-alpha hydroxylase [125], taxane 2-alpha hydroxylase [126], taxane 7-alpha hydroxylase [127], taxane 14-alpha hydroxylase [128] and phenylalanine aminomutase [129].

In addition to elucidation of the biosynthetic enzymes, progresses have been made in identification of transcription factors involved in taxol biosynthesis, which include members of the AP2 and WRKY families [130]. A recent report showed that the bHLH transcription factors of TcJAMYC1, TcJAMYC2 and TcJAMYC4 act as negative regulators of taxol biosynthesis *in T. cuspidata* cultured cells [131].

Due to the extremely low content of taxol (at ppm level) in plant, it requires massive harvesting to obtain sufficient amounts of the drug; thus, productions by total synthesis, semi-synthesis, tissue or cell cultures, endophytic fungal fermentation and more recently metabolic engineering and synthetic biology have attracted great interests [132]. Precursors of taxol biosynthesis have been produced in *Escherichia coli* [7] and *Saccharomyces cerevisiae* [123, 133], and the integration of parts (modules) of the whole pathway in separate organisms cultured together led to the combination of production of taxadiene in *E. coli* and oxygenation of taxadiene by *S. cerevisiae* [9].

3.3 Triterpenoids

Triterpenoids are cyclization product of squalene which is condensed by two molecules of FPP. In general,

triterpenoids are formed from MVA pathway in cytoplasm, as sesquiterpenoids.

3.3.1 Ginsenosides

Ginseng, the root of *Panax ginseng*, is one of the oldest traditional medicines and is widely regarded as a tonic in East Asia [88–90]. The principle bioactive constituents of Ginseng are ginsenosides, a group of tetra- or pentacyclic triterpene glycosides belonging to saponins [134]. The clinical and pharmacological activities of ginsenosides include anti-diabetic, anticancer, anti-amestic hypoglycemic, radioprotective, immunomodulatory, neuroprotective and anti-stress [135–139]. More than 40 ginsenosides have been isolated from the white and the red ginseng, and they show different biological activities based on their structural differences [140]. Generally, the major pharmacologically active ginsenosides belong to tetracyclic dammarane- and pentacyclic oleanane-type triterpene saponins [141].

The common precursor of ginsenosides is squalene, which is formed by condensation of two FPPs with squalene synthase (SS) [135, 142, 143]. In Ginseng, squalene is converted into dammarenediol-II by squalone epoxidase (SE). The cyclization of 2,3-oxidosqualene can result in two different type of triterpenoids: dammarane and oleanane type. Ginsenosides belonging to dammarane-type triterpenoids are biosynthesized from 2,3-oxidosqualene by dammarenediol synthase (DS) to form dammarenediol-II [144], whereas the biosynthesis of oleanane-type ginsenosides is started by β-amyrin synthase (PNY1) that transforms 2,3-oxidosqualene into β-amyrin [145, 146]. SS is considered a rate-limiting enzyme in the pathway and catalyzes the initial biosynthetic step for both steroids and triterpenoids [147]. PgPDR, a member of ABC transporters, was found to be involved in the ginsenosides accumulation upon MeJA induction [148].

3.3.2 Cucurbitacins

Cucurbitacins, conferring a bitter taste in cucurbits such as cucumber, melon, watermelon, squash, and pumpkin, belong to a class of highly oxidized tetracyclic triterpenoids mainly found in the plant of Cucurbitaceae family, in which *Gynostemma pentaphyllum*, *Hemsleya chinesis*, *Siraitia grosvenorii* and *Bolbostemma paniculatum* are well-known TCM plants. Recent studies suggest that cucurbitacins repress cancer cell progression [149] and inhibit neuroblastoma cell proliferation through up-regulation of PETN (phosphatase and tensin homolog) [150]. By genome-wide association study based on the genome variation map of 115 diverse cucumber lines, the gene of Csa6G088690 (Bi) encoding oxidosqualene cyclase is found to be correlated to the cucurbitacin C (CuC)

biosynthesis. Co-expression and co-regulation studies revealed a 9-gene module responsible for CucC biosynthesis, of which, four enzymes, including Bi, two P450s and one ACT, were characterized. Moreover, two bHLH transcription factors, Bl (bitter leaf) and Bt (bitter fruit), were found to directly regulate the expression of 9-gene module in cucumber leaf and fruit, respectively. During the cucumber domestication, mutations occurred within Bt promoter region which decreased its expression in the fruit tissue which may have been selected and fixed and resulted in nonbitter fruit we eat nowadays [151].

3.3.3 Glycyrrhizin

The roots and stolons of *Glycyrrhiza* plants (*G.uralensis* and *G. glabra*) contain a large amount of oleanane-type triterpenoid glycyrrhizin. It is not only used worldwide as a natural sweetener and flavoring additive due to its sweet taste, but also exhibit a wide range of pharmacological activities, including anti-inflammatory [152], immunomodulatory [153], anti-ulcer [154], anti-allergy [155], and antiviral activity [156–158].

From *G. glabra*, genes that encode enzymes responsible for triterpene skeleton formation, including the squalene synthase (SS) and β-amyrin synthase (bAS), were isolated [159, 160]. Later biosynthesis steps of glycyrrhizin involve a series of oxidative reactions at positions C-11 and C-30 and glucuronylation of the C-3 hydroxyl group. Enzymes that catalyze the oxidation steps have been found to be cytochrome P450 monooxygenases. One of them, CYP88D6, was characterized to catalyze the sequential two-step oxidation of β-amyrin at C-11 to produce 11-oxo-β-amyrin by both in vitro assay with recombinant protein and co-expression with β-amyrin synthase in yeast [161]. Another P450, CYP72A154, was identified to be responsible for three sequential oxidations at C-30 to transform 11-oxo-β-amyrin to glycyrrhetinic acid, a glycyrrhizin aglycone [162]. Both CYP88D6 and CYP72A154 transcripts were detected in the roots and stolons, but not in the leaves or stems, which is consistent with the accumulation pattern of glycyrrhizin in planta [161, 162].

4 Alkaloids

Alkaloids are a group of nitrogen-containing compounds with basic properties, most of which are derivatives of amino acids [163–166]. Biosynthesis of alkaloids usually starts from modification of amino acids, mostly decarboxylation or deamination, and undergoes further steps like methylation, hydroxylation and oxidation, and/or coupled with other compounds. There are over 12,000 alkaloids that have been identified from plants. Although widely distributed in plants, they are particularly enriched in certain families, such as Solanaceae, Manispermaceae, Papaveraceae, Berberidaceae and Fabaceae (Table 2).

It is noteworthy that the most of alkaloids display bioactivities to certain degrees, often derived from their nitrogen-containing properties. Not surprisingly, alkaloids constitute the major portion of drugs both in history and nowadays. The discovery and isolation of morphine from the opium poppy (*Papaver somniferum*) by Friedrich Sertürner in 1806 is a milestone in the history of pharmacy. Investigations of biosynthesis of natural alkaloids such as morphinan, vindoline and noscapine have been intensive and led to the complete elucidation of the pathway [167–170], and increasing alkaloid biosynthesis in plant through co-expression of enzymes genes was also reported [114]. Unfortunately, although alkaloids with TCM background like camptothecin, higenamine, huperzine A and tetrandrine have been used in pharmacy, reports of their biosynthesis are relatively rare. We list in Table 3 several

Table 2 List of examples of TCM plants rich in terpenoids

Plant species	Chinese name in Pin-yin	Family	Representative compounds
Pogostemon cablin	Guanghuoxiang	Lamiaceae	Patchoulol
Artemisia annua	Huanghuahao or Qinghao	Asteraceae	Artemisinine
Salvia miltiorrhiza	Danshen	Lamiaceae	Tanshinone
Taxus chinensis	Hongdoushan	Taxaceae	Paclitaxel
Andrographis paniculata	Chuanxinlian	Acanthaceae	Andrographolide
Isodon rubescens	Donglingcao	Lamiaceae	Oridonin, ponicidin
Isodon amethystoides	Xiangchacai	Lamiaceae	Oridonin, ponicidin
Tripterygium wilfordii	Leigongteng	Celastraceae	Triptolide
Panax ginseng	Ginsen or Renshen	Araliaceae	Ginsenosides
Panax notoginseng	Sanqi	Araliaceae	Notoginsenosides
Radix liquiritiae	Gancao	Fabaceae	Glycyrrhizin
Dioscorea polystachya	Shuyu	Dioscoreaceae	Dioscin

Table 3 List of examples of TCM plants rich in alkaloids

Plant species	Chinese name in Pin-yin	Family	Representative compounds	References
Camptotheca acuminate	Xishu	Cornaceae	Camptothecin	[171–173]
Coptis chinensis	Huanglian	Ranunculaceae	Berberine	[174, 175]
Isatis indigotica	Songlan	Brassicaceae	Isatin, indigotin	[176]
Baphicacanthus cusia	Baalan	Acanthaceae	Isatin, indigotin	[177]

typical alkaloids in TCM plants, and the relevant references. Various aspects on the alkaloid biosynthesis, regulation and metabolites trafficking can be found in review articles [178–183]. Without doubt more efforts are needed to study alkaloids in TCM plants to further explore their biological activities and facilitate their usage.

5 Perspective

Unlike model plant or staple crops, medicinal plants often lack a well-studied genetic background and a high-quality genome sequence. Due to the recently developed high-throughput sequencing technologies, it is possible to generate transcriptomic data of medicinal plants in a short time at an affordable cost. Comparative analysis of chemical constituents, transcriptomes and correlation of spatial and temporal patterns of gene expressions with those of metabolite accumulation have led to the identification of candidate genes of the biosynthesis pathway [184]. GWS combined with metabolomics analysis (mGWAS) provides a powerful platform which screens a large number of accessions simultaneously to understand genetic contributions to the metabolic diversity [185, 186].

Throughout the history, herbal plants are an integral part of our lives. In addition to curing illness, they are grown in elegant gardens, provide natural fragrance, delicate accessories and stimulate appetite. The biosynthesis of metabolites in medicinal plants is complex and specialized and involves many sequence-similar but functionally diverged enzymes. With the fast development of new technologies of analytical chemistry, bioinformatics and synthetic biology, more and more achievements will be made in this genomic or post-genomic era and bring us better life.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Harvey AL, Edrada-Ebel R, Quinn RJ (2015) The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 14:111–129
2. Qiu J (2007) Traditional medicine: a culture in the balance. Nature 448:126–128
3. Sakurai Y, Kolokoltsov AA, Chen CC et al (2015) Ebola virus. Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment. Science 347:995–998
4. Liu J, Lee J, Salazar Hernandez MA et al (2015) Treatment of obesity with celastrol. Cell 161:999–1011
5. Chan E, Tan M, Xin J et al (2010) Interactions between traditional Chinese medicines and Western therapeutics. Curr Opin Drug Discov Dev 13:50–65
6. Butelli E, Titta L, Giorgio M et al (2008) Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nat Biotechnol 26:1301–1308
7. Ajikumar PK, Xiao WH, Tyo KE et al (2010) Isoprenoid pathway optimization for Taxol precursor overproduction in *Escherichia coli*. Science 330:70–74
8. Paddon CJ, Westfall PJ, Pitera DJ et al (2013) High-level semi-synthetic production of the potent antimalarial artemisinin. Nature 496:528–532
9. Zhou K, Qiao K, Edgar S et al (2015) Distributing a metabolic pathway among a microbial consortium enhances production of natural products. Nat Biotechnol 33:377–383
10. Winkel BS (2004) Metabolic channeling in plants. Annu Rev Plant Biol 55:85–107
11. Dixon RA, Achnine L, Kota P et al (2002) The phenylpropanoid pathway and plant defence-a genomics perspective. Mol Plant Pathol 3:371–390
12. Vogt T (2010) Phenylpropanoid biosynthesis. Mol Plant 3:2–20
13. Hartmann T (2007) From waste products to ecochemicals: fifty years research of plant secondary metabolism. Phytochemistry 68:2831–2846
14. Naoumkina MA, Zhao Q, Gallego-Giraldo L et al (2010) Genome-wide analysis of phenylpropanoid defence pathways. Mol Plant Pathol 11:829–846
15. Weisshaar B, Jenkins GI (1998) Phenylpropanoid biosynthesis and its regulation. Curr Opin Plant Biol 1:251–257
16. Di P, Zhang L, Chen J et al (2013) (1)(3)C tracer reveals phenolic acids biosynthesis in hairy root cultures of *Salvia miltiorrhiza*. ACS Chem Biol 8:1537–1548
17. Wang B, Sun W, Li Q et al (2015) Genome-wide identification of phenolic acid biosynthetic genes in *Salvia miltiorrhiza*. Planta 241:711–725

18. Martens S, Mithofer A (2005) Flavones and flavone synthases. Phytochemistry 66:2399–2407

19. Williams CA, Grayer RJ (2004) Anthocyanins and other flavonoids. Nat Prod Rep 21:539–573

20. Ferrer JL, Jez JM, Bowman ME et al (1999) Structure of chalcone synthase and the molecular basis of plant polyketide biosynthesis. Nat Struct Biol 6:775–784

21. Kuo SM (1997) Dietary flavonoid and cancer prevention: evidence and potential mechanism. Crit Rev Oncog 8:47–69

22. Sutter A, Poulton J, Grisebach H (1975) Oxidation of flavanone to flavone with cell-free extracts from young parsley leaves. Arch Biochem Biophys 170:547–556

23. Martens S, Forkmann G, Matern U et al (2001) Cloning of parsley flavone synthase I. Phytochemistry 58:43–46

24. Gebhardt YH, Witte S, Steuber H et al (2007) Evolution of flavone synthase I from parsley flavanone 3beta-hydroxylase by site-directed mutagenesis. Plant Physiol 144:1442–1454

25. Gebhardt Y, Witte S, Forkmann G et al (2005) Molecular evolution of flavonoid dioxygenases in the family Apiaceae. Phytochemistry 66:1273–1284

26. Stotz G, Forkmann G (1981) Oxidation of flavanones to flavones with flower extracts of *Antirrhinum majus* (Snapdragon). Z Naturforsch C 36:737–741

27. Kitada C, Gong Z, Tanaka Y et al (2001) Differential expression of two cytochrome P450s involved in the biosynthesis of flavones and anthocyanins in chemo-varietal forms of *Perilla frutescens*. Plant Cell Physiol 42:1338–1344

28. Nakatsuka T, Nishihara M, Mishiba K et al (2005) Temporal expression of flavonoid biosynthesis-related genes regulates flower pigmentation in gentian plants. Plant Sci 168:1309–1318

29. Sasaki K, Tsurumaru Y, Yamamoto H et al (2011) Molecular characterization of a membrane-bound prenyltransferase specific for isoflavone from *Sophora flavescens*. J Biol Chem 286:24125–24134

30. Ma CJ, Li GS, Zhang DL et al (2005) One step isolation and purification of liquiritigenin and isoliquiritigenin from *Glycyrrhiza uralensis* Risch. using high-speed counter-current chromatography. J Chromatogr A 1078:188–192

31. Akashi T, Aoki T, Ayabe S (1998) Identification of a cytochrome P450 cDNA encoding (2S)-flavanone 2-hydroxylase of licorice (*Glycyrrhiza echinata* L.; Fabaceae) which represents licodione synthase and flavone synthase II. FEBS Lett 431:287–290

32. Akashi T, Aoki T, Ayabe S (1999) Cloning and functional expression of a cytochrome P450 cDNA encoding 2-hydroxyisoflavanone synthase involved in biosynthesis of the isoflavonoid skeleton in licorice. Plant Physiol 121:821–828

33. Nurul Islam M, Downey F, Ng CY (2011) Comparative analysis of bioactive phytochemicals from *Scutellaria baicalensis*, *Scutellaria lateriflora*, *Scutellaria racemosa*, *Scutellaria tomentosa* and *Scutellaria wrightii* by LC-DAD-MS. Metabolomics 7:446–453

34. Xu H, Park NI, Li X et al (2010) Molecular cloning and characterization of phenylalanine ammonia-lyase, cinnamate 4-hydroxylase and genes involved in flavone biosynthesis in *Scutellaria baicalensis*. Bioresour Technol 101:9715–9722

35. Park NI, Xu H, Li X et al (2011) Enhancement of flavone levels through overexpression of chalcone isomerase in hairy root cultures of *Scutellaria baicalensis*. Funct Integr Genom 11:491–496

36. Schneider K, Hovel K, Witzel K et al (2003) The substrate specificity-determining amino acid code of 4-coumarate: CoA ligase. Proc Natl Acad Sci USA 100:8601–8606

37. Kuzovkina IN, Guseva AV, Alterman IE et al (2001) Flavonoid production in transformed *Scutellaria baicalensis* roots and ways of its regulation. Russ J Plant Physiol 48:448–452

38. Yuan Y, Qi L, Yang J et al (2015) A Scutellaria baicalensis R2R3-MYB gene, SbMYB8, regulates flavonoid biosynthesis and improves drought stress tolerance in transgenic tobacco. Plant Cell Tissue Org 120:961–972

39. Veitch NC (2013) Isoflavonoids of the leguminosae. Nat Prod Rep 30:988–1027

40. Steele CL, Gijzen M, Qutob D et al (1999) Molecular characterization of the enzyme catalyzing the aryl migration reaction of isoflavonoid biosynthesis in soybean. Arch Biochem Biophys 367:146–150

41. Jung W, Yu O, Lau SM et al (2000) Identification and expression of isoflavone synthase, the key enzyme for biosynthesis of isoflavones in legumes. Nat Biotechnol 18:208–212

42. Shimada N, Akashi T, Aoki T et al (2000) Induction of isoflavonoid pathway in the model legume *Lotus japonicus*: molecular characterization of enzymes involved in phytoalexin biosynthesis. Plant Sci 160:37–47

43. Kim BG, Kim SY, Song HS et al (2003) Cloning and expression of the isoflavone synthase gene (IFS-Tp) from *Trifolium pratense*. Mol Cells 15:301–306

44. Shao BM, Xu W, Dai H et al (2004) A study on the immune receptors for polysaccharides from the roots of *Astragalus membranaceus*, a Chinese medicinal herb. Biochem Biophys Res Commun 320:1103–1111

45. Matkovic Z, Zivkovic V, Korica M et al (2010) Efficacy and safety of *Astragalus membranaceus* in the treatment of patients with seasonal allergic rhinitis. Phytother Res 24:175–181

46. Xu Q, Ma X, Liang X (2007) Determination of astragalosides in the roots of *Astragalus* spp. using liquid chromatography tandem atmospheric pressure chemical ionization mass spectrometry. Phytochem Anal 18:419–427

47. Wang D, Zhuang Y, Tian Y et al (2012) Study of the effects of total flavonoids of *Astragalus* on atherosclerosis formation and potential mechanisms. Oxid Med Cell Longev 2012:282383

48. Hirotani M, Zhou Y, Rui H et al (1994) Cycloartane triterpene glycosides from the hairy root cultures of *Astragalus membranaceus*. Phytochemistry 37:1403–1407

49. Cho H-J, Widholm J (2002) Improved shoot regeneration protocol for hairy roots of the legume *Astragalus sinicus*. Plant Cell Tissue Org 69:259–269

50. Duan LX, Chen TL, Li M et al (2012) Use of the metabolomics approach to characterize Chinese medicinal material Huangqi. Mol Plant 5:376–386

51. Lukaczer D, Darland G, Tripp M et al (2005) Clinical effects of a proprietary combination isoflavone nutritional supplement in menopausal women: a pilot trial. Altern Ther Health Med 11:60–65

52. Rong H, Stevens JF, Deinzer ML et al (1998) Identification of isoflavones in the roots of *Pueraria lobata*. Planta Med 64:620–627

53. Ohshima Y, Okuyama T, Takahashi K et al (1988) Isolation and high performance liquid chromatography (HPLC) of isoflavonoids from the Pueraria root. Planta Med 54:250–254

54. He X, Blount JW, Ge S et al (2011) A genomic approach to isoflavone biosynthesis in kudzu (*Pueraria lobata*). Planta 233:843–855

55. Chen G, Wu X, Zhou WL et al (2010) Preparation and assay of C-glucosyltransferase from roots of *Pueraria lobata*. J Environ Biol 31:655–660

56. Yazaki K, Sasaki K, Tsurumaru Y (2009) Prenylation of aromatic compounds, a key diversification of plant secondary metabolites. Phytochemistry 70:1739–1745

57. Kitaoka M, Kadokawa H, Sugano M et al (1998) Prenylflavonoids: a new class of non-steroidal phytoestrogen (Part 1). Isolation of 8-isopentenylnaringenin and an initial study on its structure-activity relationship. Planta Med 64:511–515

58. Sasaki K, Mito K, Ohara K et al (2008) Cloning and characterization of naringenin 8-prenyltransferase, a flavonoid-specific prenyltransferase of *Sophora flavescens*. Plant Physiol 146:1075–1084

59. Yu L, Li H, Huang G et al (1992) Clinical observations on treatment of 120 cases of coronary heart disease with herba epimedii. J Tradit Chin Med 12:30–34

60. Li C, Li Q, Mei Q et al (2015) Pharmacological effects and pharmacokinetic properties of icariin, the major bioactive component in *Herba Epimedii*. Life Sci 126:57–68

61. Islam NM, Yoo HH, Lee MW et al (2008) Simultaneous quantitation of five flavonoid glycosides in Herba Epimedii by high-performance liquid chromatography-tandem mass spectrometry. Phytochem Anal 19:71–77

62. Zeng S, Xiao G, Guo J et al (2010) Development of a EST dataset and characterization of EST-SSRs in a traditional Chinese medicinal plant, *Epimedium sagittatum* (Sieb. Et Zucc.) Maxim. BMC Genom 11:94

63. Huang W, Zeng S, Xiao G et al (2015) Elucidating the biosynthetic and regulatory mechanisms of flavonoid-derived bioactive components in *Epimedium sagittatum*. Front Plant Sci 6:689

64. Zhou L, Zuo Z, Chow MS (2005) Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol 45:1345–1359

65. Zhang HS, Wang SQ (2006) Salvianolic acid B from *Salvia miltiorrhiza* inhibits tumor necrosis factor-alpha (TNF-alpha)-induced MMP-2 upregulation in human aortic smooth muscle cells via suppression of NAD(P)H oxidase-derived reactive oxygen species. J Mol Cell Cardiol 41:138–148

66. Wu WY, Wang YP (2012) Pharmacological actions and therapeutic applications of *Salvia miltiorrhiza* depside salt and its active components. Acta Pharmacol Sin 33:1119–1130

67. Eberle D, Ullmann P, Werck-Reichhart D et al (2009) cDNA cloning and functional characterisation of CYP98A14 and NADPH:cytochrome P450 reductase from *Coleus blumei* involved in rosmarinic acid biosynthesis. Plant Mol Biol 69:239–253

68. Gershenzon J, Dudareva N (2007) The function of terpene natural products in the natural world. Nat Chem Biol 3:408–414

69. Chen F, Tholl D, Bohlmann J et al (2011) The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. Plant J 66:212–229

70. Oldfield E, Lin FY (2012) Terpene biosynthesis: modularity rules. Angew Chem Int Ed 51:1124–1137

71. Cooperative Research Group on Artemisinin Structure (1977) Artemisinin—a new sesquiterpene lactone. Chin Sci Bull 22:142

72. Liu JM, Ni MY, Fan JF et al (1979) Structure and reaction of Artemisinin. Acta Chim Sin 37:129–143

73. Miller LH, Su X (2011) Artemisinin: discovery from the Chinese herbal garden. Cell 146:855–858

74. Schramek N, Wang H, Romisch-Margl W et al (2010) Artemisinin biosynthesis in growing plants of *Artemisia annua*. A $^{13}CO_2$ study. Phytochemistry 71:179–187

75. Mercke P, Bengtsson M, Bouwmeester HJ et al (2000) Molecular cloning, expression, and characterization of amorpha-4,11-diene synthase, a key enzyme of artemisinin biosynthesis in *Artemisia annua* L. Arch Biochem Biophys 381:173–180

76. Teoh KH, Polichuk DR, Reed DW et al (2006) *Artemisia annua* L. (Asteraceae) trichome-specific cDNAs reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin. FEBS Lett 580:1411–1416

77. Zhang Y, Teoh KH, Reed DW et al (2008) The molecular cloning of artemisinic aldehyde Delta 11(13) reductase and its role in glandular trichome-dependent biosynthesis of artemisinin in Artemisia annua. J Biol Chem 283:21501–21508

78. Teoh KH, Polichuk DR, Reed DW et al (2009) Molecular cloning of an aldehyde dehydrogenase implicated in artemisinin biosynthesis in *Artemisia annua*. Botany 87:635–642

79. Afrin S, Huang J-J, Luo Z-Y (2015) JA-mediated transcriptional regulation of secondary metabolism in medicinal plants. Sci Bull 60:1062–1072

80. Yu ZX, Li JX, Yang CQ et al (2012) The jasmonate-responsive AP2/ERF transcription factors AaERF1 and AaERF2 positively regulate artemisinin biosynthesis in *Artemisia annua* L. Mol Plant 5:353–365

81. Ma D, Pu G, Lei C et al (2009) Isolation and characterization of AaWRKY1, an *Artemisia annua* transcription factor that regulates the amorpha-4,11-diene synthase gene, a key gene of artemisinin biosynthesis. Plant Cell Physiol 50:2146–2161

82. Graham IA, Besser K, Blumer S et al (2010) The genetic map of *Artemisia annua* L. identifies loci affecting yield of the antimalarial drug artemisinin. Science 327:328–331

83. Ma DM, Wang Z, Wang L et al (2015) A genome-wide scenario of terpene pathways in self-pollinated *Artemisia annua* L. Mol Plant. doi:10.1016/j.molp.2015.07.004

84. Swamy MK, Sinniah UR (2015) A comprehensive review on the phytochemical constituents and pharmacological activities of *Pogostemon cablin* benth: an aromatic medicinal plant of industrial importance. Molecules 20:8521–8547

85. Munck SL, Croteau R (1990) Purification and characterization of the sesquiterpene cyclase patchoulol synthase from *Pogostemon cablin*. Arch Biochem Biophys 282:58–64

86. Deguerry F, Pastore L, Wu S et al (2006) The diverse sesquiterpene profile of patchouli, *Pogostemon cablin*, is correlated with a limited number of sesquiterpene synthases. Arch Biochem Biophys 454:123–136

87. Yu ZX, Wang LJ, Zhao B et al (2015) Progressive regulation of sesquiterpene biosynthesis in Arabidopsis and Patchouli (*Pogostemon cablin*) by the miR156-targeted SPL transcription factors. Mol Plant 8:98–110

88. Court WE (2000) Ginseng: the history of an insignificant plant. Pharm Hist (Lond) 30:38–44

89. Sathiyamoorthy S, In JG, Gayathri S et al (2010) Gene ontology study of methyl jasmonate-treated and non-treated hairy roots of *Panax ginseng* to identify genes involved in secondary metabolic pathway. Russ J Genet 46:932–939

90. Zhu GY, Li YW, Hau DK et al (2011) Protopanaxatriol-type ginsenosides from the root of *Panax ginseng*. J Agric Food Chem 59:200–205

91. Liu CX, Yin QQ, Zhou HC et al (2012) Adenanthin targets peroxiredoxin I and II to induce differentiation of leukemic cells. Nat Chem Biol 8:486–493

92. Zhen T, Wu CF, Liu P et al (2012) Targeting of AML1-ETO in t(8;21) leukemia by oridonin generates a tumor suppressor-like protein. Sci Transl Med 4:127ra38

93. Shamon LA, Pezzuto JM, Graves JM et al (1997) Evaluation of the mutagenic, cytotoxic, and antitumor potential of triptolide, a highly oxygenated diterpene isolated from *Tripterygium wilfordii*. Cancer Lett 112:113–117

94. Li YI, Elmer G, Leboeuf RC (2008) Tanshinone IIA reduces macrophage death induced by hydrogen peroxide by upregulating glutathione peroxidase. Life Sci 83:557–562

95. Dong Y, Morris-Natschke SL, Lee KH (2011) Biosynthesis, total syntheses, and antitumor activity of tanshinones and their analogs as potential therapeutic agents. Nat Prod Rep 28:529–542

96. Gao W, Hillwig ML, Huang L et al (2009) A functional genomics approach to tanshinone biosynthesis provides stereochemical insights. Org Lett 11:5170–5173

97. Kai GY, Liao P, Xu H et al (2012) Molecular mechanism of elicitor-induced tanshinone accumulation in *Salvia miltiorrhiza* hairy root cultures. Acta Physiol Plant 34:1421–1433

98. Guo J, Zhou YJ, Hillwig ML et al (2013) CYP76AH1 catalyzes turnover of miltiradiene in tanshinones biosynthesis and enables heterologous production of ferruginol in yeasts. Proc Natl Acad Sci USA 110:12108–12113

99. Cui G, Duan L, Jin B et al (2015) Functional divergence of diterpene syntheses in the medicinal plant *Salvia miltiorrhiza* Bunge. Plant Physiol. doi:10.1104/pp.15.00695

100. Yan Y, Wang Z, Tian W et al (2010) Generation and analysis of expressed sequence tags from the medicinal plant *Salvia miltiorrhiza*. Sci China Life Sci 53:273–285

101. Wenping H, Yuan Z, Jie S et al (2011) De novo transcriptome sequencing in *Salvia miltiorrhiza* to identify genes involved in the biosynthesis of active ingredients. Genomics 98:272–279

102. Ma Y, Yuan L, Wu B et al (2012) Genome-wide identification and characterization of novel genes involved in terpenoid biosynthesis in *Salvia miltiorrhiza*. J Exp Bot 63:2809–2823

103. Yang L, Ding G, Lin H et al (2013) Transcriptome analysis of medicinal plant *Salvia miltiorrhiza* and identification of genes related to tanshinone biosynthesis. PLoS One 8:e80464

104. Xu Z, Peters RJ, Weirather J et al (2015) Full-length transcriptome sequences and splice variants obtained by a combination of sequencing platforms applied to different root tissues of *Salvia miltiorrhiza* and tanshinone biosynthesis. Plant J 82:951–961

105. Xu YH, Wang JW, Wang S et al (2004) Characterization of GaWRKY1, a cotton transcription factor that regulates the sesquiterpene synthase gene (+)-delta-cadinene synthase-A. Plant Physiol 135:507–515

106. Murata J, Roepke J, Gordon H et al (2008) The leaf epidermome of *Catharanthus roseus* reveals its biochemical specialization. Plant Cell 20:524–542

107. Wang H, Penmetsa RV, Yuan M et al (2012) Development and characterization of BAC-end sequence derived SSRs, and their incorporation into a new higher density genetic map for cultivated peanut (*Arachis hypogaea* L.). BMC Plant Biol 12:10

108. Li JT, Dong JE, Liang ZS et al (2008) Distributional difference of fat-soluble compounds in the roots, stems and leaves of four Salvia plants. Fen Zi Xi Bao Sheng Wu Xue Bao 41:44–52

109. Zhang C, Yan Q, Cheuk WK et al (2004) Enhancement of tanshinone production in *Salvia miltiorrhiza* hairy root culture by Ag+ elicitation and nutrient feeding. Planta Med 70:147–151

110. Ge X, Wu J (2005) Induction and potentiation of diterpenoid tanshinone accumulation in *Salvia miltiorrhiza* hairy roots by beta-aminobutyric acid. Appl Microbiol Biotechnol 68:183–188

111. Ge X, Wu J (2005) Tanshinone production and isoprenoid pathways in *Salvia miltiorrhiza* hairy roots induced by Ag$^+$ and yeast elicitor. Plant Sci 168:487–491

112. Shi M, Kwok KW, Wu JY (2007) Enhancement of tanshinone production in *Salvia miltiorrhiza* Bunge (red or Chinese sage) hairy-root culture by hyperosmotic stress and yeast elicitor. Biotechnol Appl Biochem 46:191–196

113. Yan Q, Wu J, Liu R (2011) Modeling of tanshinone synthesis and phase distribution under the combined effect of elicitation and in situ adsorption in *Salvia miltiorrhiza* hairy root cultures. Biotechnol Lett 33:813–819

114. Hao X, Shi M, Cui L et al (2015) Effects of methyl jasmonate and salicylic acid on tanshinone production and biosynthetic gene expression in transgenic *Salvia miltiorrhiza* hairy roots. Biotechnol Appl Biochem 62:24–31

115. Schiff PB, Fant J, Horwitz SB (1979) Promotion of microtubule assembly in vitro by taxol. Nature 277:665–667

116. Hefner J, Ketchum RE, Croteau R (1998) Cloning and functional expression of a cDNA encoding geranylgeranyl diphosphate synthase from *Taxus canadensis* and assessment of the role of this prenyltransferase in cells induced for taxol production. Arch Biochem Biophys 360:62–74

117. Wildung MR, Croteau R (1996) A cDNA clone for taxadiene synthase, the diterpene cyclase that catalyzes the committed step of taxol biosynthesis. J Biol Chem 271:9201–9204

118. Walker K, Schoendorf A, Croteau R (2000) Molecular cloning of a taxa-4(20),11(12)-dien-5alpha-ol-O-acetyl transferase cDNA from *Taxus* and functional expression in *Escherichia coli*. Arch Biochem Biophys 374:371–380

119. Walker K, Croteau R (2000) Taxol biosynthesis: molecular cloning of a benzoyl-CoA: taxane 2alpha-O-benzoyltransferase cDNA from *Taxus* and functional expression in *Escherichia coli*. Proc Natl Acad Sci USA 97:13591–13596

120. Walker K, Fujisaki S, Long R et al (2002) Molecular cloning and heterologous expression of the C-13 phenylpropanoid side chain-CoA acyltransferase that functions in Taxol biosynthesis. Proc Natl Acad Sci USA 99:12715–12720

121. Walker K, Croteau R (2000) Molecular cloning of a 10-deacetylbaccatin III-10-O-acetyl transferase cDNA from *Taxus* and functional expression in Escherichia coli. Proc Natl Acad Sci USA 97:583–587

122. Walker K, Long R, Croteau R (2002) The final acylation step in taxol biosynthesis: cloning of the taxoid C13-side-chain N-benzoyltransferase from *Taxus*. Proc Natl Acad Sci USA 99:9166–9171

123. Jennewein S, Long RM, Williams RM et al (2004) Cytochrome P450 taxadiene 5alpha-hydroxylase, a mechanistically unusual monooxygenase catalyzing the first oxygenation step of taxol biosynthesis. Chem Biol 11:379–387

124. Schoendorf A, Rithner CD, Williams RM et al (2001) Molecular cloning of a cytochrome P450 taxane 10 beta-hydroxylase cDNA from *Taxus* and functional expression in yeast. Proc Natl Acad Sci USA 98:1501–1506

125. Jennewein S, Rithner CD, Williams RM et al (2001) Taxol biosynthesis: taxane 13 alpha-hydroxylase is a cytochrome P450-dependent monooxygenase. Proc Natl Acad Sci USA 98:13595–13600

126. Chau M, Croteau R (2004) Molecular cloning and characterization of a cytochrome P450 taxoid 2alpha-hydroxylase involved in Taxol biosynthesis. Arch Biochem Biophys 427:48–57

127. Chau M, Jennewein S, Walker K et al (2004) Taxol biosynthesis: molecular cloning and characterization of a cytochrome P450 taxoid 7 beta-hydroxylase. Chem Biol 11:663–672

128. Jennewein S, Rithner CD, Williams RM et al (2003) Taxoid metabolism: taxoid 14beta-hydroxylase is a cytochrome P450-dependent monooxygenase. Arch Biochem Biophys 413:262–270

129. Walker KD, Klettke K, Akiyama T et al (2004) Cloning, heterologous expression, and characterization of a phenylalanine aminomutase involved in Taxol biosynthesis. J Biol Chem 279:53947–53954

130. Li S, Zhang P, Zhang M et al (2013) Functional analysis of a WRKY transcription factor involved in transcriptional activation of the DBAT gene in *Taxus chinensis*. Plant Biol 15:19–26

131. Lenka SK, Nims NE, Vongpaseuth K et al (2015) Jasmonate-responsive expression of paclitaxel biosynthesis genes in *Taxus cuspidata* cultured cells is negatively regulated by the bHLH transcription factors TcJAMYC1, TcJAMYC2, and TcJAMYC4. Front Plant Sci 6:115

132. Ajikumar PK, Tyo K, Carlsen S et al (2008) Terpenoids: opportunities for biosynthesis of natural product drugs using engineered microorganisms. Mol Pharm 5:167–190

133. Hefner J, Rubenstein SM, Ketchum RE et al (1996) Cytochrome P450-catalyzed hydroxylation of taxa-4(5),11(12)-diene to taxa-4(20),11(12)-dien-5alpha-ol: the first oxygenation step in taxol biosynthesis. Chem Biol 3:479–489

134. Shibata S (2001) Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. J Korean Med Sci 16(Suppl):S28–S37

135. Wang BX, Zhou QL, Yang M et al (2003) Hypoglycemic activity of ginseng glycopeptide. Acta Pharmacol Sin 24:50–54

136. Sritularak B, Morinaga O, Yuan CS et al (2009) Quantitative analysis of ginsenosides Rb1, Rg1, and Re in American ginseng berry and flower samples by ELISA using monoclonal antibodies. J Nat Med 63:360–363

137. Xie JT, Wang CZ, Zhang B et al (2009) In vitro and in vivo anticancer effects of American ginseng berry: exploring representative compounds. Biol Pharm Bull 32:1552–1558

138. Joo KM, Lee JH, Jeon HY et al (2010) Pharmacokinetic study of ginsenoside Re with pure ginsenoside Re and ginseng berry extracts in mouse using ultra performance liquid chromatography/mass spectrometric method. J Pharm Biomed Anal 51:278–283

139. Cho IH (2012) Effects of *Panax ginseng* in neurodegenerative diseases. J Ginseng Res 36:342–353

140. Chan PC, Huff J (2012) Hexane fraction of American ginseng suppresses colitis and colon cancer. Cancer Prev Res (Phila) 5:982

141. Cao H, Nuruzzaman M, Xiu H et al (2015) Transcriptome analysis of methyl jasmonate-elicited *Panax ginseng* adventitious roots to discover putative ginsenoside biosynthesis and transport genes. Int J Mol Sci 16:3035–3057

142. Sathiyamoorthy S, In JG, Gayathri S et al (2010) Generation and gene ontology based analysis of expressed sequence tags (EST) from a *Panax ginseng* C. A. Meyer roots. Mol Biol Rep 37:3465–3472

143. Kim YJ, Jeon JN, Jang MG et al (2014) Ginsenoside profiles and related gene expression during foliation in *Panax ginseng* Meyer. J Ginseng Res 38:66–72

144. Tansakul P, Shibuya M, Kushiro T et al (2006) Dammarenediol-II synthase, the first dedicated enzyme for ginsenoside biosynthesis, in *Panax ginseng*. FEBS Lett 580:5143–5149

145. Luo HM, Song JY, Li XY et al (2013) Cloning and expression analysis of a key device of HMGR gene involved in ginsenoside biosynthesis of *Panax ginseng* via synthetic biology approach. Yao Xue Xue Bao 48:219–227

146. Han JY, Kwon YS, Yang DC et al (2006) Expression and RNA interference-induced silencing of the dammarenediol synthase gene in *Panax ginseng*. Plant Cell Physiol 47:1653–1662

147. Lee MH, Jeong JH, Seo JW et al (2004) Enhanced triterpene and phytosterol biosynthesis in *Panax ginseng* overexpressing squalene synthase gene. Plant Cell Physiol 45:976–984

148. Zhang R, Huang J, Zhu J et al (2013) Isolation and characterization of a novel PDR-type ABC transporter gene PgPDR3 from *Panax* ginseng C. A. Meyer induced by methyl jasmonate. Mol Biol Rep 40:6195–6204

149. Gao Y, Islam MS, Tian J et al (2014) Inactivation of ATP citrate lyase by Cucurbitacin B: a bioactive compound from cucumber, inhibits prostate cancer growth. Cancer Lett 349:15–25

150. Shang Y, Guo XX, Li WW et al (2014) Cucurbitacin-B inhibits neuroblastoma cell proliferation through up-regulation of PTEN. Eur Rev Med Pharmacol Sci 18:3297–3303

151. Shang Y, Ma Y, Zhou Y et al (2014) Plant science. Biosynthesis, regulation, and domestication of bitterness in cucumber. Science 346:1084–1088

152. Matsui S, Matsumoto H, Sonoda Y et al (2004) Glycyrrhizin and related compounds down-regulate production of inflammatory chemokines IL-8 and eotaxin 1 in a human lung fibroblast cell line. Int Immunopharmacol 4:1633–1644

153. Takahara T, Watanabe A, Shiraki K (1994) Effects of glycyrrhizin on hepatitis B surface antigen: a biochemical and morphological study. J Hepatol 21:601–609

154. He JX, Akao T, Nishino T et al (2001) The influence of commonly prescribed synthetic drugs for peptic ulcer on the pharmacokinetic fate of glycyrrhizin from Shaoyao–Gancao–Tang. Biol Pharm Bull 24:1395–1399

155. Park HY, Park SH, Yoon HK et al (2004) Anti-allergic activity of 18beta-glycyrrhetinic acid-3-O-beta-D-glucuronide. Arch Pharm Res 27:57–60

156. Ito M, Nakashima H, Baba M et al (1987) Inhibitory effect of glycyrrhizin on the in vitro infectivity and cytopathic activity of the human immunodeficiency virus [HIV (HTLV-III/LAV)]. Antivir Res 7:127–137

157. Ito M, Sato A, Hirabayashi K et al (1988) Mechanism of inhibitory effect of glycyrrhizin on replication of human immunodeficiency virus (HIV). Antivir Res 10:289–298

158. Cinatl J, Morgenstern B, Bauer G et al (2003) Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. Lancet 361:2045–2046

159. Hayashi H, Hirota A, Hiraoka N et al (1999) Molecular cloning and characterization of two cDNAs for *Glycyrrhiza glabra* squalene synthase. Biol Pharm Bull 22:947–950

160. Hayashi H, Huang P, Kirakosyan A et al (2001) Cloning and characterization of a cDNA encoding beta-amyrin synthase involved in glycyrrhizin and soyasaponin biosyntheses in licorice. Biol Pharm Bull 24:912–916

161. Seki H, Ohyama K, Sawai S et al (2008) Licorice beta-amyrin 11-oxidase, a cytochrome P450 with a key role in the biosynthesis of the triterpene sweetener glycyrrhizin. Proc Natl Acad Sci USA 105:14204–14209

162. Seki H, Sawai S, Ohyama K et al (2011) Triterpene functional genomics in licorice for identification of CYP72A154 involved in the biosynthesis of glycyrrhizin. Plant Cell 23:4112–4123

163. Bush LP, Wilkinson HH, Schardl CL (1997) Bioprotective alkaloids of grass-fungal endophyte symbioses. Plant Physiol 114:1–7

164. Laurent P, Braekman JC, Daloze D et al (2002) In vitro production of adaline and coccinelline, two defensive alkaloids from ladybird beetles (Coleoptera: Coccinellidae). Insect Biochem Mol Biol 32:1017–1023

165. Kajii S, Nishikawa T, Isobe M (2008) Synthetic studies and biosynthetic speculation on marine alkaloid chartelline. Chem Commun 3121–3123

166. Joosten L, van Veen JA (2011) Defensive properties of pyrrolizidine alkaloids against microorganisms. Phytochem Rev 10:127–136

167. Winzer T, Gazda V, He Z et al (2012) A Papaver somniferum 10-gene cluster for synthesis of the anticancer alkaloid noscapine. Science 336:1704–1708

168. Winzer T, Kern M, King AJ et al (2015) Morphinan biosynthesis in opium poppy requires a P450-oxidoreductase fusion protein. Science 349:309–312

169. Qu Y, Easson ML, Froese J et al (2015) Completion of the seven-step pathway from tabersonine to the anticancer drug precursor vindoline and its assembly in yeast. Proc Natl Acad Sci USA 112:6224–6229

170. Lau W, Sattely ES (2015) Six enzymes from mayapple that complete the biosynthetic pathway to the etoposide aglycone. Science 349:1224–1228

171. Sun Y, Luo H, Li Y et al (2011) Pyrosequencing of the *Camptotheca acuminata* transcriptome reveals putative genes involved in camptothecin biosynthesis and transport. BMC Genom 12:533

172. Lorence A, Medina-Bolivar F, Nessler CL (2004) Camptothecin and 10-hydroxycamptothecin from *Camptotheca acuminata* hairy roots. Plant Cell Rep 22:437–441

173. Valletta A, Trainotti L, Santamaria AR et al (2010) Cell-specific expression of tryptophan decarboxylase and 10-hydroxygeraniol oxidoreductase, key genes involved in camptothecin biosynthesis in *Camptotheca acuminata* Decne (Nyssaceae). BMC Plant Biol 10:69

174. Mao T, Yan Q, Zhou F et al (1997) Isolation and identification of berberine from cell cultures of *Coptis chinensis*. Chin J Biotechnol 13:193–199

175. Liu XF, Xia YM, Fang Y (2005) Effect of metal ions on the interaction between bovine serum albumin and berberine chloride extracted from a traditional Chinese Herb *Coptis chinensis* franch. J Inorg Biochem 99:1449–1457

176. Zou P, Koh HL (2007) Determination of indican, isatin, indirubin and indigotin in Isatis indigotica by liquid chromatography/electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 21:1239–1246

177. Xiaojing W, Liang Z, Chen HB et al (2013) Identification of Daqingye and Banlangen including crude drugs and decoction dregs from three plant species by normal light and fluorescence microscopy. Microsc Res Tech 76:774–782

178. Ziegler J, Facchini PJ (2008) Alkaloid biosynthesis: metabolism and trafficking. Annu Rev Plant Biol 59:735–769

179. Cordell GA (2013) Fifty years of alkaloid biosynthesis in Phytochemistry. Phytochemistry 91:29–51

180. Yamada Y, Sato F (2013) Transcription factors in alkaloid biosynthesis. Int Rev Cell Mol Biol 305:339–382

181. Panjikar S, Stoeckigt J, O'Connor S et al (2012) The impact of structural biology on alkaloid biosynthesis research. Nat Prod Rep 29:1176–1200

182. Schafer H, Wink M (2009) Medicinally important secondary metabolites in recombinant microorganisms or plants: progress in alkaloid biosynthesis. Biotechnol J 4:1684–1703

183. Facchini PJ (2006) Regulation of alkaloid biosynthesis in plants. Alkaloids Chem Biol 63:1–44

184. Goossens A (2015) It is easy to get huge candidate gene lists for plant metabolism now, but how to get beyond? Mol Plant 8:2–5

185. Chan EK, Rowe HC, Hansen BG et al (2010) The complex genetic architecture of the metabolome. PLoS Genet 6:e1001198

186. Riedelsheimer C, Lisec J, Czedik-Eysenberg A et al (2012) Genome-wide association mapping of leaf metabolic profiles for dissecting complex traits in maize. Proc Natl Acad Sci USA 109:8872–8877