Research progress on the biosynthesis, metabolic engineering, and pharmacology of bioactive compounds from the *Lonicera* genus

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Abstract

The *Lonicera* genus with medicinal and edible characteristics is a rich source of bioactive compounds that together account for numerous health benefits. Plants within this genus have been used traditionally in China to treat heat-related illnesses, viral respiratory infections, skin diseases, and inflammation for thousands of years. The main bioactive compounds include chlorogenic acids, flavonoids, iridoids, and triterpene saponins. Aside from the role of this genus as a valuable medicinal resource, constituent members have also been used in the food and cosmetic industries. Due to the huge demand for plants within the *Lonicera* genus, and the propensity for overexploitation of the wild resources of *Lonicera*, there is a need to meet this demand *via* alternative and efficient strategies such as metabolic engineering and green production. Here, we provide a comprehensive review of relevant scientific literature covering the structure, biosynthesis, metabolic engineering, pharmacology and phylogenetic analysis of the main bioactive constituents of the *Lonicera* genus. Finally, we proffer suggestions on the prospects of fully exploiting and utilizing plants of the *Lonicera* genus as useful medicinal plant resources.

Introduction

The *Lonicera* Linn. genus is a constituent member of the Caprifoliaceae family[1]. It is the largest genus in this family and comprises at least 200 species with a notable presence in North Africa, North America, Asia, and Europe[1]. Members of the *Lonicera* genus possess a wide range of economic benefits from their use as ornamental plants to food and as plants credited with numerous health benefits. Conspicuous among the numerous members of this genus with known medicinal uses are *L. japonica*, *L. macranthoides*, *L. hypoglauca*, *L. confusa*, and *L. fulvotomentosa*[2]. Though these species feature prominently in the Chinese Pharmacopeia, other species such as *L. acuminata*, *L. buchananii*, and *L. similis* are recognized as medicinal resources in certain parts of China[1]. Among the aforementioned species, *L. japonica* takes precedence over the rest due to its high medicinal and nutritional value[3,4]. For instance, the microRNA MIR2911, an isolate from *L. japonica*, has been reported to inhibit the replication of viruses[5–7]. Also, the water extract of *L. japonica* has been used to produce various beverages and health products[8]. The *Lonicera* genus therefore possesses huge prospects in the pharmaceutical, food, and cosmetic industries as an invaluable raw material[9].

The main active constituents of the *Lonicera* genus include organic acids, flavonoids, iridoids, and triterpene saponins. Chlorogenic acids, iridoids, and flavones are mainly credited with the anti-inflammatory, antiviral, anticancer, and antioxidant effects of the various *Lonicera* species[10–13]. Their hepatoprotective, immune modulatory, anti-tumor and anti-Alzheimer's effects are for the most part ascribed to the triterpene saponins[14–16]. As stated in the Chinese Pharmacopoeia and backed by the findings of diverse research groups, the plants of the *Lonicera* genus are known to possess high amounts of organic acids (specifically chlorogenic acid) and pentacyclic triterpenoid saponins[2,17–19]. The flower and flower bud have traditionally served as the main medicinal parts of the *Lonicera* genus even though there is ample evidence that the leaves possess the same chemical composition[20]. A perusal of the current scientific literature reveals the fact that little attention has been devoted to exploring the biosynthesis of the chemical constituents of the *Lonicera* genus with the view to finding alternative means of obtaining higher yields. It is therefore imperative that priority is given to the exploration of the biosynthesis of these bioactive compounds as a possible means of resource protection. There is also the need for further research on ways to fully tap the medicinal benefits of other plant parts in the *Lonicera* genus.

Here, we provide a comprehensive review of relevant scientific literature covering the structure, pharmacology, multi-omics analyses, phylogenetic analysis, biosynthesis, and metabolic engineering of the main bioactive constituents of the *Lonicera* genus. Finally, we proffer suggestions on the prospects of fully exploiting and utilizing plants of the *Lonicera* genus as useful medicinal plant resources.

Structure and distribution of main bioactive constituents of *Lonicera*

A total of at least 400 secondary metabolites have been reported for the *Lonicera* genus. These metabolites are categorized into four main groups (Fig. 1a), including not less than 50 organic acids, 80 flavonoids, 80 iridoids, and 80 triterpene saponins[21−23]. Organic acids are mainly derivatives of p-hydroxycinnamic acid and quinic acid. Among the organic acids, chlorogenic acids are reported to be the main bioactive compounds in *L. japonica*[24−26]. The organic acids are most abundant in the leaves, while the least amounts are found in the stem of *L. japonica*. The flowers of the plant are known to contain moderately high amounts of organic acids[27]. The basic core structure of the flavonoids is 2-phenylchromogen. Luteolin and its glycoside which are characteristic flavonoids of the *Lonicera* genus are most abundant in *L. japonica*[28]. On the whole, the flavonoid contents in *L. japonica* are also highest in the leaves, available in moderate amounts in the flowers, and in lowest amounts in the stem[21]. The core structures of the iridoids are iridoid alcohols, the chemical properties of which are similar to hemiacetal. The iridoids often exist in the form of iridoid glycosides in plants. Secoiridoids glycosides are predominant in the *Lonicera* genus[25]. In *L. japonica*, the contents of the iridoids are most abundant in the flowers, moderate in leaves, and lowest in the stem[21]. The characteristic saponins of the *Lonicera* genus are mainly pentacyclic triterpenoids, including the hederin-, oleanane-, lupane-, ursulane- and fernane-types, etc[22]. The hederin-type saponins are reported in the highest amounts in *L. macranthoides*[17] (Fig. 1b).

Biosynthesis and regulation of the main bioactive constituents of *Lonicera*

Biosynthesis of chlorogenic acids and flavonoids

The similarities between chlorogenic acid (CGA) and flavonoids can be traced back to their biosynthesis since p-coumaroyl CoA serves as the common precursor for these compounds[29]. p-coumaroyl CoA is obtained through sequential catalysis of phenylalanine and its biosynthetic intermediates by phenylalanine-ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H) and 4-coumarate CoA ligase (4CL)[30−33].

CGA is a phenolic acid composed of caffeic acid and quinic acid and is the most important bioactive compound among the organic acids. Its biosynthesis has been relatively well-established; three main biosynthetic routes have been propounded (Fig. 2a). One route relates to the catalysis of caffeoyl-CoA and quinic acid by hydroxycinnamoyl-CoA quinate transferase (HQT)/hydroxycinnamoyl CoA shikimate/quinate hydroxycinnamoyl transferase (HCT) to produce CGA[34−37]. The HQT-mediated pathway has been deemed the major route for CGA synthesis in in different plant species[38,39]. The second biosynthetic route stems from the biosynthesis of p-coumaroyl quinate through the catalytic effect of HCT/HQT and subsequent hydroxylation of p-coumaroyl quinate under the catalysis of p-coumarate 3'-hydroxylase (C3'H)[34,36,37]. For the third route, caffeoyl glucoside serves as the intermediate to form CGA, a process that is catalyzed by hydroxycinnamyl D-glucose: quinic acid hydroxycinnamyl transferase (HCGQT)[40,41].

The key enzymes in the biosynthesis of p-coumaroyl CoA, and invariably CGA, thus, PAL, C4H, and 4CL have been established in diverse studies such as enzyme gene overexpression/knockdown[42], enzyme activity analysis[33] and transcriptomics[18]. However, the centrality of HQT in the biosynthesis of CGA remains disputable. While some studies have reported a strong correlation between HQT expression level with CGA content and distribution[18,35,39,43,44], others found no such link[45], bringing into question the role of HQT as a key enzyme in CGA biosynthesis.

Few studies have been conducted on the regulation of CGA biosynthesis in the *Lonicera* genus. It was found that overexpression of the transcription factor, LmMYB15 in *Nicotiana benthamiana* can promote CGA accumulation by directly activating 4CL or indirectly binding to MYB3 and MYB4
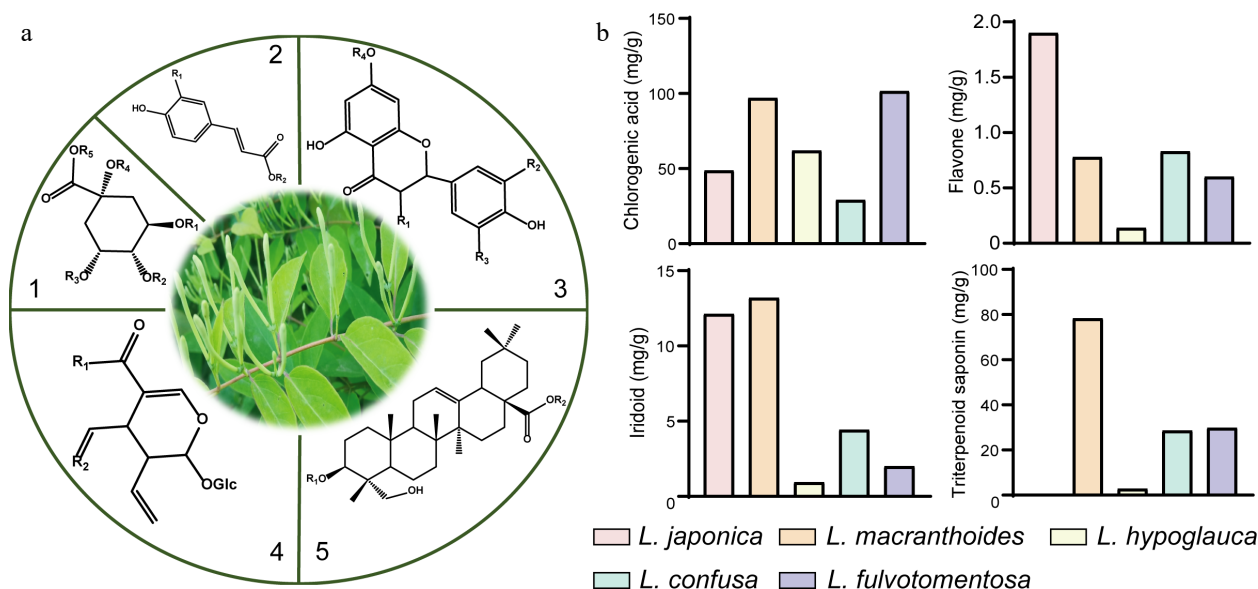

Fig. 1 Core structures of main secondary metabolites and their distribution in five species of *Lonicera*. (a) 1 and 2, the main core structures of organic acids; 3, the main core structures of flavonoids; 4, the main core structures of iridoids; 5, the main core structures of triterpene saponins. (b) Comparison of dry weight of four kinds of substances in five species of *Lonicera*[17,28].
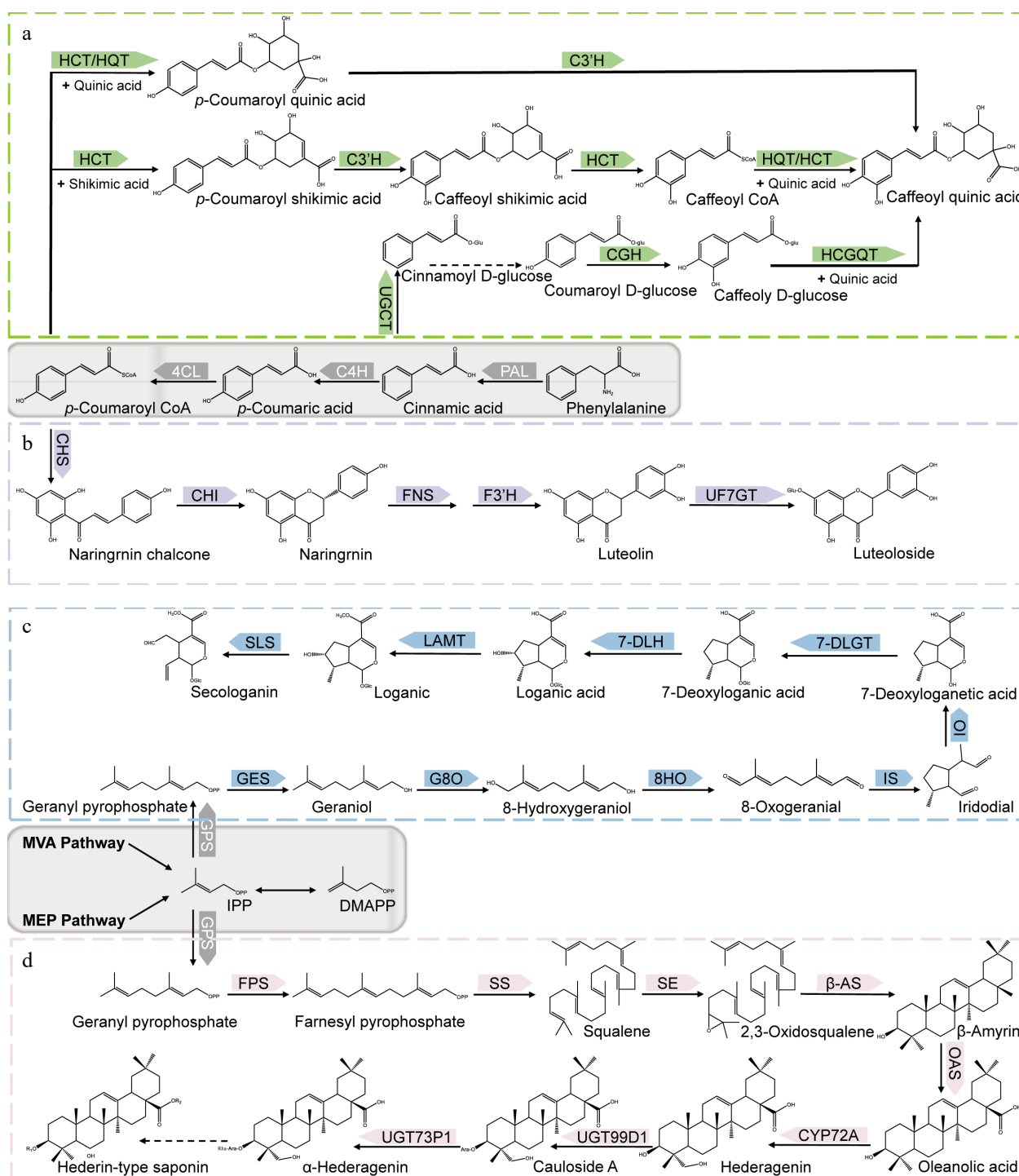
Fig. 2 Biosynthetic pathways of main bioactive constituents of *Lonicera*. (a) Biosynthetic pathways of chlorogenic acid. (b) Biosynthetic pathways of luteoloside. (c) Biosynthetic pathways of secologanin. (d) Biosynthetic pathways of hederin-type triterpene saponins. PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-hydroxycinnamoyl CoA ligase; HCT, hydroxycinnamoyl CoA shikimate/quinate hydroxycinnamoyl transferase; C3'H, p-coumaroyl 3-hydroxylase; HQT, hydroxycinnamoyl-CoA quinate transferase; UGCT, UDP glucose: cinnamate glucosyl transferase; CGH, p-coumaroyl-D-glucose hydroxylase; HCGQT, hydroxycinnamoyl D-glucose: quinate hydroxycinnamoyl transferase; CHS, Chalcone synthase; CHI, Chalcone isomerase; FNS, Flavone synthase; F3H, flavonoid 30-monooxygenase/flavonoid 30-hydroxylase; UF7GT, flavone 7-O-β-glucosyltransferase; GPS, Geranyl pyrophosphatase; GES, geraniol synthase; G8O, geraniol 10-hydroxylase/8-oxidase; 8HO, 8-hydroxygeraniol oxidoreductase; IS, iridoid synthase; IO, iridoid oxidase; 7DLGT, 7-deoxyloganetic acid glucosyltransferase; 7DLH, 7-deoxyloganic acid hydroxylase; LAMT, loganic acid O-methyltransferase; SLS, secologanin synthase; FPS, farnesyl pyrophosphate synthase; SS, squalene synthase; SE, squalene epoxidase; β-AS, β-amyrin synthase; OAS, oleanolic acid synthetase.

promoters[46]. LjbZIP8 can specifically bind to PAL2 and act as a transcriptional repressor to reduce PAL2 expression levels and CGA content[47]. Under NaCl stress, increased PAL expression promoted the accumulation of phenolic substances in leaves without oxidative damage, a condition that was conducive to the accumulation of the bioactive compounds in leaves[48].

Luteolin and its derivative luteolin 7-O- glucoside (luteoloside) are representative flavonoids of the *Lonicera* genus. Similar to CGA, luteolin is biosynthesized from *p*-coumaroyl CoA but *via* a different route. The transition from *p*-coumaroyl CoA to luteolin is underpinned by sequential catalysis by chalcone synthetase (CHS), chalcone isomerase (CHI), flavonoid synthetase (FNS), and flavonoid 3'-monooxygenase/flavonoid 3'-hydroxylase (F3'H)[45,49,50] (Fig. 2b). Luteoloside is synthesized from luteolin by UDP glucose-flavonoid 7-O-β-glucosyltransferase (UF7GT)[51]. Similar to CGA biosynthesis, the key enzymes of luteolin synthesis include PAL, C4H, and 4CL in addition to FNS[33,45,52]. The content of luteoloside was found to be highly abundant in senescing leaves relative to other tissues such as stem, flowers, and even young leaves[52]. Through transcriptomic analysis, luteoloside biosynthesis-related differentially expressed unigenes (DEGs) such as PAL2, C4H2, flavone 7-O-β-glucosyltransferase (UFGT), 4CL, C4H, chalcone synthase 2 and flavonoid 3'-monooxygenase (F3'H) genes were found to be upregulated in the senescing leaves. Biosynthesis-related transcription factors such as MYB, bHLH, and WD40 were also differentially expressed during leaf senescence[52], while bHLH, ERF, MYB, bZIP, and NAC were differentially expressed during flower growth[53]. Further analysis of the transcription factors revealed that MYB12, MYB44, MYB75, MYB114, MYC12, bHLH113, and TTG1 are crucial in luteoloside biosynthesis[52,53].

Biosynthesis of iridoids and triterpene saponins

The biosynthesis of terpenoids mainly involves three stages; formation of intermediates, formation of basic structural skeleton, and modification of basic skeleton[54]. The intermediates of terpenoids are mainly formed through the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways, and eventually converted to the universal isoprenoid precursors, isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP) through a series of enzyme-catalyzed reactions. Under the catalysis of geranyl pyrophosphatase (GPS), IPP is then converted to geranyl pyrophosphate (GPP). Different terpenoids are subsequently derived from GPP as the intermediate product. For instance, in the formation of secoiridoid, GPP first removes the phosphoric acid group to obtain geraniol, second through a series of reactions such as oxidation and cyclization, the skeleton of iridoid, namely iridodial, can be obtained. Finally, through a series of reactions, the basic carbon skeleton of the secoiridoid, namely secologanin, is obtained[55–61] (Fig. 2c). In the formation of triterpene saponins, the key step lies in the formation of the precursor, 2,3-oxidosqualene, a reaction that is catalyzed by squalene epoxidase (SE). There are many pentacyclic triterpenes in the *Lonicera* genus, the most important type being the hederin-type saponins with hederagenin as aglycones. Hederin-type saponins are produced after the synthesis of oleanolic acid from β-amyrin and catalyzed by β-starch synthetase (β-AS) and Oleanolic acid synthase (OAS)[62,63]. The skeletal modification of the triterpenoid saponins is mainly achieved *via* the activities of the CYP450 enzymes and UDP-glycosyltransferase (UGT).

Hence, the corresponding aglycones are first obtained *via* oxidation by the CYP450 enzymes (e.g., CYP72A), and further subjected to glycosylation by the appropriate UGT enzyme[63–65] (Fig. 2d). Skeletal formations of the iridoids and triterpene saponins in general have been well researched, but the same cannot be said about the enzymes involved in biosynthesis of these groups of compounds in the *Lonicera* genus. To fully utilize the iridoids and triterpene saponins in the *Lonicera* genus, it is necessary to further explore their biosyntheses with the view to enhancing and optimizing the process.

Metabolic engineering of the main bioactive compounds of the Lonicera genus

Given the importance of the bioactive compounds in the *Lonicera* genus, continual isolation of these compounds using the traditional methods are not only tedious and time-consuming, but also unsustainable. With the development and application of microbial metabolic engineering, different strategies have been introduced to produce these bioactive compounds by heterologous synthesis (Table 1).

Metabolic engineering of chlorogenic acid, luteolin and their glycosides

Due to the demand for CGA in the food, pharmaceutical, chemical, and cosmetic industries, the traditional means of obtaining the same requires a relatively longer period for plant maturation to obtain low yields of the desired product. This therefore brings into question the sustainability and efficiency of this approach. The alternative and sustainable approach has been to produce CGA using synthetic biology and metabolic engineering.

Current research has sought to utilize *Escherichia coli* (and its mutant strain) and *Saccharomyces cerevisiae* to synthetically generate CGA and other flavonoids[66–73]. For instance, Cha et al. employed two strains of *E. coli* to produce a relatively good yield of CGA (78 mg/L). Their approach was based on the ability of one strain to generate caffeic acid from glucose and the other strain to use the caffeic acid produced and quinic acid as starting materials to synthesize CGA[66]. Using a bioengineered mutant of *E. coli* (aroD mutant), Kim et al. increased the yield of CGA to as high as 450 mg/L[67]. Others have sought to increase the yield of CGA by employing a polyculture of three *E. coli* strains that act as specific modules for the *de novo* biosynthesis of caffeic acid, quinic acid and CGA. This strategy eliminates the competition posed by the precursor of CGA (i.e., caffeic acid and quinic acid) and generally results in improved production of CGA[68]. *Saccharomyces cerevisiae* is a chassis widely used for the production of natural substances from plants with an intimal structure that can be used for the expression of cytochrome P450 enzymes that cannot be expressed in *E. coli*. Researchers have used yeast to increase the production of organic acids[69]. A *de novo* biosynthetic pathway for the construction of CGA in yeast has been reported new cell-free biosynthetic system based on a mixture of chassis cell extracts and purified Spy cyclized enzymes were adopted by Niu et al. to a produce the highest yield of CGA reported so far up to 711.26 ± 15.63 mg/L[70].

There are many studies on the metabolic engineering for the synthesis of flavonoids, but few on luteolin and its glycosides. Strains of *E. coli* have been engineered with specific uridine

Table 1. Biosynthesis of *Lonicera*-specialized metabolites using metabolic engineering.

Engineering bacteria	Operational methods	Products	Yield	Refs
S. cerevisiae	Eliminate the tyrosine-induced feedback inhibition, delete genes involved in competing pathways and overexpress rate-limiting enzymes	Caffeic acid	569.0 mg/L	[69]
S. cerevisiae	Employ a heterologous tyrosine ammonia lyase and a 4HPA3H complex composed of HpaB and HpaC derived from different species	Caffeic acid	289.4 mg/L	[73]
S. cerevisiae	Supply and recycle of three cofactors: FADH2, S-adenosyl-L-methion, NADPH	Caffeic acid Ferulic acid	Caffeic acid: 5.5 g/L; Ferulic acid: 3.8 g/L	[117]
E. coli	Knocking out competing pathways	Caffeic acid	7,922 mg/L	[118]
E. coli	Artificial microbial community, a polyculture of three recombinant *Escherichia coli* strains	Chlorogenic acid	250 μM	[68]
Cell-free biosynthesis	Extract and purify spy-cyclized enzymes (CFBS-mixture)	Chlorogenic acid	711.26 mg/L	[70]
S. cerevisiae	Three metabolic engineering modules were systematically optimized: shikimate pathway and carbon distribution, branch pathways, CGA pathway genes	Chlorogenic acid	Flask fermentation: 234.8 mg/L; Fed-batch fermentation: 806.8 mg/L	[119]
E. coli	Using modular coculture engineering: construction of the defective strain improves the production and utilization of precursor substances	Chlorogenic acid	131.31 mg/L	[122]
E. coli	Introduce heterologous UDP-glucose biosynthetic genes	Luteolin	34 mg/L	[120]
Y. lipolytica	Overexpression of the key genes involved in the mevalonate pathway, the gene encoding cytochrome P450 (CYP716A12) to that encoding NADPH-P450 reductase	Oleanolic acid	129.9 mg/L	[85]
S. cerevisiae	Improve the pairing efficiency between Cytochrome P450 monooxygenase and reductase and the expression level of key genes	Oleanolic acid	606.9 mg/L	[121]
S. cerevisiae	Heterologous expression and optimization of CrAS, CrAO, and AtCPR1, and regulation of ERG1 and NADPH regeneration system	Oleanolic acid	433.9 mg/L	[123]

diphosphate (UDP)-dependent glycosyltransferase (UGT) to synthesize three novel flavonoid glycosides. These glycosides were quercetin 3-O-(*N*-acetyl) quinovosamine (158.3 mg/L), luteolin 7-O-(*N*-acetyl) glucosaminuronic acid (172.5 mg/L) and quercetin 3-O-(*N*-acetyl)-xylosamine (160.8 mg/L)[71]. Since most of the flavonoid glycosides synthesized in *E. coli* are glucosylated, Kim et al. in their bid to synthesize luteolin-7-O-glucuronide, deleted the araA gene that encodes UDP-4-deoxy-4-formamido-*L*-arabinose formyltransferase/UDP-glucuronic acid C-4" decarboxylase in *E.coli* and were able to obtain a yield of 300 mg/L of the desired product[72].

Metabolic engineering of iridoids and triterpene saponins

Terpenoidal saponins are mostly derived from slow-growing plants and usually possess multiple chiral centers[74]. Traditional isolation and even chemical synthesis of the terpenoidal saponins are both tedious and uneconomical for large-scale production. Therefore, it is necessary to find other ways to synthesize these compounds known to have diverse pharmacological functions.

Heterologous synthesis has become an important way to improve the target products. With the development of synthetic biology, heterologous synthesis of triterpene saponins involves chassis of both plant and microbial origin. In this regard, *Nicotiana benthamiana* is a model plant species for the reconstruction of the biosynthetic pathways of different bioactive compounds including monoterpenes, hemiterpenes, and diterpenes[59,75–77]. Aside from *Nicotiana benthamiana*, other plants have also been used as heterologous hosts[78]. Heterologous synthesis using microbial hosts mainly involves *Saccharomyces cerevisiae* and *Escherichia coli*[79–81], and other microorganisms[82,83]. Comparatively, plants as biosynthetic hosts have the advantages of an established photosynthetic system, abundant supply of relevant enzymes, and presence of cell compartments, etc. They are however not as fast growing as the microorganisms, and it is also difficult to extract and separate the desired synthesized compounds from them as hosts.

Although heterologous synthesis has many advantages, the premise of successful construction of synthetic pathway in host is to elucidate the unique structure of the compound and the key enzyme reaction mechanism in the biosynthetic pathway. There is little research on metabolic engineering of the hederin-type pentacyclic triterpene saponins in *Lonicera*, but there are studies on the heterologous synthesis of its aglycone precursor, oleanolic acid[84,85]. There is a dearth of scientific literature on key enzymes in the biosynthesis of pentacyclic triterpenoid saponins in the *Lonicera* genus.

Pharmacological activities of the main bioactive compounds of *Lonicera*

Scientific evidence by diverse research groups has linked members of the *Lonicera* genus to a wide range of pharmacological effects (Fig. 3). These pharmacological effects are elicited by different chemical constituents, much of the underlying mechanisms of which have been elucidated by the omics techniques. Here, we summarize the pharmacological effects and pharmacodynamics of the *Lonicera* genus in the last 6 years.

Anti-inflammatory effects

Bioactive compounds of plants in the *Lonicera* genus have demonstrated varying degrees of anti-inflammatory actions. In a recent study, Lv et al. showed that lonicerin inhibits the activation of NOD-like receptor thermal protein domain associated protein 3 (NLRP3) through regulating EZH2/AtG5-mediated autophagy in bone marrow-derived macrophages of C57BL/6 mice[86]. The polysaccharide extract of *L. japonica* reduces
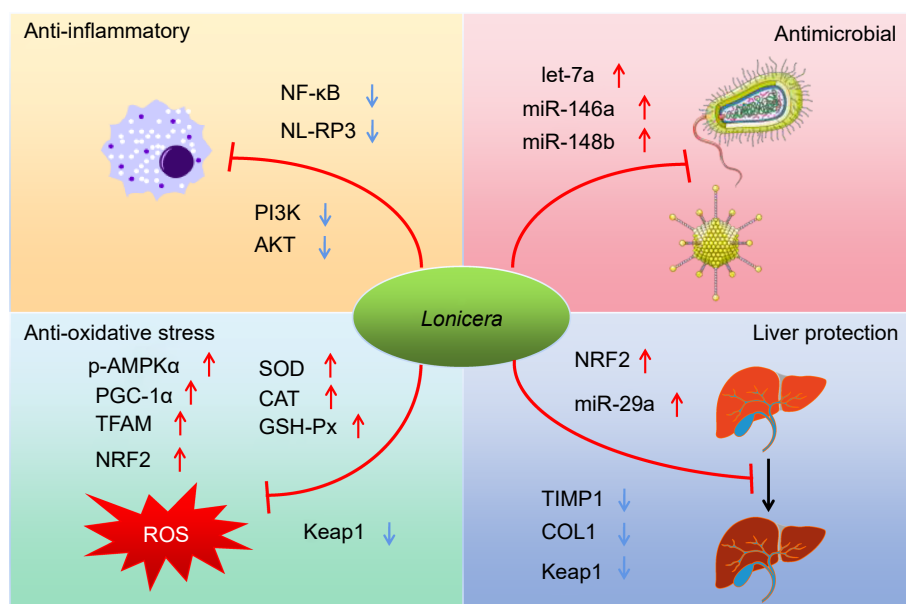
Fig. 3 Schematic summary of four main pharmacological effects (anti-inflammatory, antimicrobial, anti-oxidative and hepatoprotective effects) of the *Lonicera* genus and the underlying mechanisms of actions.

atopic dermatitis in mice by promoting Nrf2 activation and NLRP3 degradation through p62[87]. Several products of *Lonicera* have been reported to have ameliorative effects on DSS-induced colitis. Among them, flavonoids of *L. rupicola* can improve the ulcerative colitis of C57BL/6 mice by inhibiting PI3K/AKT, and pomace of *L. japonica* can improve the ulcerative colitis of C57BL/6 mice by improving the intestinal barrier and intestinal flora[88,89]. The flavonoids can also ameliorate ulcerative colitis induced by local enema of 2,4,6-trinitrobenzene sulfonic acid (TNBS) in Wistar rats by inhibiting NF-κB pathway[90]. Ethanol extract from *L. Japonica* has demonstrated the potential to inhibit the expressions of inflammatory cytokines in serum and macrophages of LPS-induced ICR mice[91]. The water extract of *L. japonica* and luteolin were found to exhibit their anti-inflammatory effects *via* the inhibition of the JAK/STAT1/3-dependent NF-κB pathway and induction of HO-1 expression in RAW263.7 cells induced by pseudorabies virus (PRV)[92].

Antimicrobial effects

Existing scientific evidence indicates that the extracts of plants in the *Lonicera* genus exhibit strong inhibition against different pathogenic microorganisms. Phenolic compounds from *L. japonica* demonstrated a particularly significant inhibitory effect against *Staphylococcus aureus* and *Escherichia coli, in vitro*, making these compounds potential food preservatives[93]. Influenza A virus is a serious threat to human health. Recent research has found the ethanol extract of *L. japonica* to possess a strong inhibitory effect against H1N1 influenza virus-infected MDCK cells and ICR mice[94]. The incidence of the COVID-19 pandemic called to action various scientists in a bid to find safe and efficacious treatment[95]. Traditional Chinese medicines became an attractive alternative in this search. The water extract of the flower bud of *L. japonica* which has traditionally served as a good antipyretic and antitussive agent attracted the attention of researchers. Scientific evidences have confirmed that the water extract of *L. japonica* can induce let-7a expression in human rhabdomyosarcoma cells or neuronal cells

and blood of lactating mice, inhibiting the entry and replication of the virus *in vitro* and *in vivo*[96]. In addition, the water extract of *L. japonica* also inhibits the fusion of human lung cancer cells Calu-3 expressing ACE2 receptor and BGK-21 cells transfected with SARS-CoV-2 spike protein, and up-regulates the expression of miR-148b and miR-146a[97].

Anti-oxidative stress effect

Oxidative stress has been implicated in the pathophysiology of many diseases, hence, amelioration of the same could be a good therapeutic approach[98,99]. In keeping with this therapeutic strategy, various compounds from the *Lonicera* genus have demonstrated the ability to relieve oxidative stress due to their pronounced antioxidant effects. For instance, the polyphenolic extract of *L. caerulea* berry was found to activate the expression of AMPK-PGC1α-NRF1-TFAM proteins in the skeletal muscle mitochondria, improve the activity of SOD, CAT and GSH-Px enzymes in blood and skeletal muscle, relieve exercise fatigue in mice by reducing oxidative stress in skeletal muscle, and enhance mitochondrial biosynthesis and cell proliferation[100]. The diverse health benefits of the anthocyanins from *L. japonica* have been mainly credited to their antioxidant and anti-inflammatory effects. The anthocyanin and cyanidin-3-o-glucoside have been reported to possess the potential to prolong life and delay senescence of *Drosophila* through the activation of the KEAP1/NRF2 signaling pathway[101].

Hepatoprotection

The liver is an essential organ that contributes to food digestion and detoxification of the body. These functions expose the liver to diverse toxins and metabolites. The *Lonicera* genus is rich in phytochemicals that confer protection on the liver against various toxins. The phenolic compound, 4, 5-di-O-Caffeoylquinic acid methyl ester was shown to be able to improve H_2O_2-induced liver oxidative damage in HepG2 cells by targeting the Keap1/Nrf2 pathway[102]. Hepatic fibrosis is a complex dynamic process, with the propensity to progress to

liver cancer in severe cases. The *L. japonicae* flos water extract solution increased the cell viability of FL83B cells treated with thioacetamide (TAA), decreased the levels of serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP), inhibited the transformation growth factor β1 (TGF-β1) and liver collagen deposition[103]. Sweroside, a secoiridoid glucoside isolate of *L. japonica* is known to protect the C57BL/6 mice liver from hepatic fibrosis by up-regulating miR-29a and inhibiting COL1 and TIMP1[104].

Other pharmacological effects

Aside from the aforementioned, other pharmacological effects have been ascribed to the *Lonicera* genus. The ethanolic extract of *L. caerulea* has been reported to inhibit the proliferation of SMMC-7721 and H22 hepatoma cells, while its anthocyanins induced the apoptosis of tumor cells *via* the release of cytochrome C and activation of caspase[105]. AMPK/PPARα axes play an important role in lipid metabolism. A chlorogenic acid-rich extract of *L. Japonica* was found to significantly decrease the early onset of high-fat diet-induced diabetes in Sprague-Dawley rats *via* the CTRPs-AdipoRs-AMPK/PPARα axes[106]. In a high-fat diet-induced non-alcoholic fatty liver disease in C57BL/6 mice, treatment with *L. caerulea* polyphenol extract

decreased serum inflammatory factors and endotoxin levels and the Firmicutes/Bacteroidetes ratio, an indication of its modulatory effect on the gut microbiota[107]. The iridoid-anthocyanin extract from *L. caerulea* berry contributed to alleviating the symptoms of intestinal infection with spirochaeta in mice[108].

Chloroplast genomic and phylogenetic relationships of *Lonicera* species

The traditional classification of the *Lonicera* genus based on the morphology of member plants is further categorized into two subgenera, *Chamaecerasus* and *Periclymenum*. The Chamaecerasus includes four categories, *Coeloxylosteum*, *Isika*, *Isoxylosteum* and *Nintooa*. The *Periclymenum* includes two categories, *Subsect. Lonicera* and *Subsect. Phenianthi* (Supplemental Table S1).

High-throughput chloroplast genome sequencing of *L. japonica* found its length to be 155078 bp, which is similar to the structure of the typical angiosperm chloroplast genome. It contains a pair of inverted repeat regions (IRa and IRb, 23774 bp), a large single copy region (LSC, 88858 bp) and a small
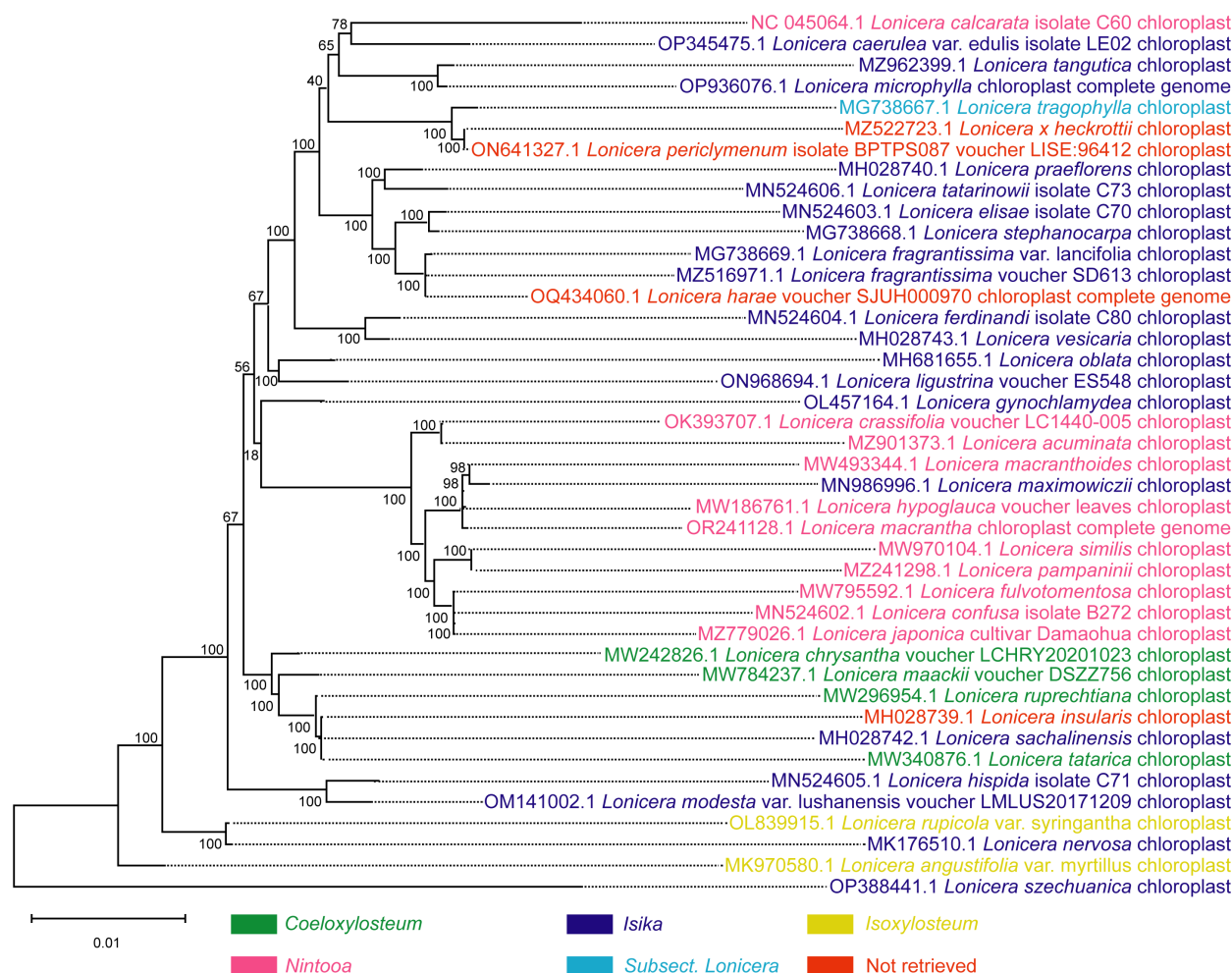

Fig. 4 Phylogenetic tree of 42 species of the *Lonicera* genus based on complete chloroplast genome sequence data. The phylogenetic tree was constructed by the maximum likelihood method. *Coeloxylosteum*, *Isika*, *Isoxylosteum*, and *Nintooa* belong to *Chamaecerasus* and *Subsect. Lonicera* belongs to the *Periclymenum*. *Chamaecerasus* and *Periclymenum* are the two subgenera of *Lonicera*. 'Not retrieved' indicates that the species failed to retrieve a subordinate taxon in the *Lonicera*.

single copy area (SSC, 18672 bp)[109,110]. However, compared with chloroplast genomes of other plants, the chloroplast genome of *L. japonica* has a unique rearrangement between trnI-CAU and trnN-GUU[110]. Based on the phylogenetic analysis of the plastid genomes of seven plants in the *Lonicera* genus, 16 diverging hot spots were identified as potential molecular markers for the development of the *Lonicera* plants[111]. The phylogeny of *Lonicera* is rarely researched at the molecular level and the pattern of repetitive variation and adaptive evolution of the genome sequence is still unknown. Chloroplast genome sequences are highly conserved, but insertions and deletions, inversions, substitutions, genome rearrangements, and translocations also occur and have become powerful tools for studying plant phylogeny[112,113].

We present here the phylogenetic tree of the *Lonicera* genus based on the published complete chloroplast genome sequences downloaded from the National Center for Biotechnology Information (NCBI) database using the Maximum likelihood method (Fig. 4). Based on our chloroplast phylogenies, we propose to merge *L. harae* into *Sect. Isika* and *L. insularis* into *Chamaecerasus*, but whether *L. insularis* belongs to *Sect. Isika* or *Sect. Coeloxylosteum* is uncertain. Based on protein-coding regions (CDS) of the chloroplast genome or complete chloroplast genomes, Liu et al. and Chen et al. supported the classification of the two subgenera in *Lonicera*[111,114]. Sun et al. and Srivastav et al. demonstrated a classification between the two subgenera with more species by using sequences of nuclear loci generated, chloroplast genome, and restriction site-associated DNA sequencing (RADSeq)[115,116]. However, our phylogenetic analysis and that of Sun et al. show relations within the subgenus *Chamaecerasus* are tanglesome in some respects[116]. Plant traits are affected by the environment to varying degrees. Since evidence of plant speciation is implicit in its genome sequence, comparative analysis at the molecular level provides a relatively accurate depiction of inherent changes that might have occurred over time. These findings suggest the need for more species of the *Lonicera* genus to be sequenced to provide a more accurate theoretical basis for the evolution of the *Lonicera* plants and a more effective revision in the classification of the *Lonicera* genus.

Conclusions and future perspectives

The *Lonicera* genus is rich in diverse bioactive compounds with immeasurable prospects in many fields. Members of this genus have been used for thousands of years in traditional Chinese medicine for heat-clearing and detoxification. These plants generally have a good taste and form part of the ingredients of various fruit juices. In cosmetics, they are known to possess anti-aging and moisturizing functions. Plants of the *Lonicera* genus are also known for their good ecological adaptability and can be used to improve soil and ecological environment. Based on the value of the *Lonicera* genus, besides researching their use through molecular biological means, their efficient utilization can also be promoted in the following ways: (1) The stems and leaves of the plants could be developed for consumption and use since the chemical profiles of these parts do not differ significantly from the flowers. This way, the wastage of this scarce resource could be minimized or avoided. (2) Most of the *Lonicera* plants are vines or shrubs and their natural regeneration speed is slow, so the introduction and

domestication of species could be strengthened to avoid over-exploitation of wild resources.

At present, only the research on the biosynthesis and efficacy of chlorogenic acid is quite comprehensive and has been used widely in various fields. There is limited research on various aspects of other bioactive compounds and should therefore be given priority in future research goals. Currently, the multi-omics analytical approach has gradually evolved as a reliable and helpful analytical platform. Hence, multi-omics research on the *Lonicera* genus could lead to discoveries in drug discovery and human health.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design, draft manuscript preparation: Yin X, Chen X, Li W, Tran LSP, Lu X; manuscript revision: Yin X, Chen X, Li W, Tran LSP, Lu X, Chen X, Yin X, Alolga RN; data/literature collection: Chen X, Yin X; figure preparation: Chen X, Yin X; figure revision: Alolga RN, Yin X, Chen X, Li W, Tran LSP, Lu X. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information file.

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Conflict of interest

The authors declare that they have no conflict of interest. Xiaojian Yin is the Editorial Board member of *Medicinal Plant Biology* who was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and the research groups.

Dates

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