



Mini-review

Microbial fortification of pharmacological metabolites in medicinal plants

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ABSTRACT

Medicinal plants are rich in secondary metabolites with beneficial pharmacological effects. The production of plant secondary metabolites is subjected to the influences by environmental factors including the plant-associated microbiome, which is crucial to the host's fitness and survival. As a result, research interests are increasing in exploiting microbial capacities for enhancing plant production of pharmacological metabolites. A growing body of recent research provides accumulating evidence in support of developing microbe-based tools for achieving this objective. This mini review presents brief summaries of recent studies on medicinal plants that demonstrate microbe-augmented production of pharmacological terpenoids, polyphenols, and alkaloids, followed by discussions on some key questions beyond the promising observations. Explicit molecular insights into the underlying mechanisms will enhance microbial applications for metabolic fortification in medicinal plants.

1. Introduction

Plants produce a huge array of organic compounds, which can be classified into primary metabolites, secondary metabolites, and hormones, based on their assumed functions [1]. Secondary metabolites refer to the subset of metabolome that are not directly involved in basic functions of growth, development, and reproduction of the organisms, but can be important for long-term survival [2]. Plant secondary metabolites (PSMs) play crucial roles in plant defense, communication, and adaptation to the environment [3], such as the pathogen-induced sulfur-containing phytoalexins that function in plant defense [4], and the antioxidant polyphenols that function in plant acclimation to UV-B [5]. PSMs are mostly categorized as polyphenols, terpenoids, and alkaloids, based on their chemical structures and biosynthetic pathways [6]. Many plants produce PSMs with beneficial pharmacological effects, including therapeutic properties, on humans and other animals, and are therefore defined as medicinal plants, such as *Artemisia annua* L. that produces the anti-malaria artemisinin and *Taxus brevifolia* that produce the anti-cancer taxol widely used for clinical applications.

Plant production of secondary metabolites is subjected to the influences by many environmental factors, in addition to the intrinsic regulation by developmental programs [7]. Plants are naturally

inhabited by a variety of epiphytic and endophytic microbes, which are collectively termed plant microbiome. While some microbes have no observable effects on plants, others can be either pathogens that cause detrimental effects on plants or beneficial species that promote plant growth and stress resistance [8,9]. On one hand, the assembly of plant microbiome is shaped by the host via integrated regulation of morphological structures, exudation of secondary metabolites, and innate immune responses [10–14]. On the other hand, the complex community of plant-associated microbes has been shown to be important for plant fitness, and is therefore suggested as an important target for agricultural and horticultural managements [15–18].

Methods of enhancing plant production of pharmacological metabolites are of great importance and of intense interests. Given the importance of microbiome to plants, research interests are increasing regarding potential roles of plant-associated microbes in the production of secondary metabolites in medicinal plants [19,20]. This mini review focuses on microbial fortification of pharmacological metabolites in medicinal plants. In addition to the emerging evidence that showed microbe-enhanced production of terpenoids that are mainly exemplified by mono-, di-, tri-, and tetra-terpenes, an overview of microbial influences on medicinal plants is provided regarding flavonoids and non-flavonoid polyphenols as well as alkaloids. Finally, we discuss key

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questions in this emerging research field including potential mechanisms underlying the beneficial effects, which will underpin microbial applications for metabolic fortification in medicinal plants.

2. Microbe-increased production of terpenoids

As the largest and structurally most diverse group of natural compounds, terpenes and their terpenoid derivatives (hereafter used interchangeably for simplicity) play major and various roles among the pharmacological PSMs [21,22]. Based on the number of their 5-carbon isoprene units, most terpenoids are subgrouped into hemi- (C_5), mono- (C_{10}), sesqui- (C_{15}), di- (C_{20}), sester- (C_{25}), tri- (C_{30}), tetra- (C_{40}), and polyterpenoids ($C_{>40}$) [23,24]. Recent studies of medicinal plants have highlighted important roles of microbes in the production of terpenoids, which are mainly exemplified by mono-, di-, tri-, and tetraterpenes (Table 1).

2.1. Monoterpenes

Microbe-enhanced production of monoterpenes was recently identified as a major contributor to the geo-herbalism of Citri Reticulatae Pericarpium (CRP) [25], which is the dried ripe peel of *Citrus reticulata* Blanco or its cultivars and is used for clinical treatment of indigestion and inflammatory syndromes of the respiratory tract [25,26]. Terpenes, known with antioxidant and antimicrobial activities, account for the majority of the essential oils that are important to the pharmacological quality of CRP [25,27]. CRP quality had been known to be influenced by the citrus planting area, but how such influence occurs was unclear. Su et al. examined the essential oils of CRPs from *C. reticulata* grown in different geo-regions. A group of seven monoterpenes, including o-cymene, β -myrcene, α -pinene, β -pinene, δ -carene, α -thujene and α -terpinene, were identified as biomarkers that predicted the citrus planting area with an average accuracy of 82.86 %. Eight bacteria genera from the *C. reticulata* rhizosphere microbiome were then identified as showing significant positive correlations with five of these biomarkers. The microbial influences on monoterpene production were subsequently confirmed in *C. reticulata* treated with an isolated *Streptomyces* strain. Interestingly, a combination of this *Streptomyces* strain and an endophytic *Serratia* strain somewhat further increase the production of α -pinene, β -pinene and α -thujene in *C. reticulata* leaves, whereas the treatment with the *Serratia* strain alone showed similar levels of monoterpene production as the control plants [25], suggesting that the microbial enhancement of *C. reticulata* monoterpene production in the natural environment is an outcome from integrative actions at the microbiome level. As expected, the microbiome features are tightly correlated with the soil properties in the different geo-regions [25]. Similarly, geographic specificity was observed for both the microbiome and the sesquiterpenoid contents of *Atractylodes macrocephala* Koidz., which is a herbaceous medicinal plant used in traditional Chinese medicine formulations to invigorate the spleen [28]. The microbial enhancement of *C. reticulata* monoterpene production was attributed to the higher expression levels of some genes related to terpenoid biosynthesis and modifications [25].

In addition to rhizosphere microbiome, endophytic microbial community can also be correlated with monoterpene production in medicinal plants, as shown in a recent study of the *Gentiana* species *G. officinalis* and *G. siphonantha* that are collectively known as *Gentiana* sect. *Cruciata* plants [29,30]. *Gentiana* sect. *Cruciata* possesses anti-inflammatory, antifungal, antihistamine and antihepatotoxic activities, mainly due to the monoterpenes including gentiopicroside, loganic acid, swertiamarine and sweroside [29–31]. In *G. officinalis* and *G. siphonantha* collected from the same experimental field, the content of loganic acid was positively correlated with endophytic fungi of *Tetracladium*, *unidentified Ascomycota* sp and *unidentified Sebaciales* sp, while the contents of gentiopicroside, swertiamarine and sweroside were positively correlated with endophytic bacteria *Polyangium* [30].

Table 1

| Examples of medicinal plants in which pharmacological PSMs were enhanced by microbial treatments or correlated with (*) the plant microbiome.

Metabolites	Microbes	Plant tissue	Plant species	Reference
Monoterpene				
α -pinene	<i>Streptomyces</i>	Leaves	<i>Citrus reticulata</i>	[25]
β -pinene	Strep-4		'Chachi'	
α -thujene				
β -myrcene				
Loganic acid	<i>Tetracladium</i> (fungus)	Roots	<i>Gentiana officinalis</i>	[30]*
Gentiopicroside, Swertiamarine, Sweroside	<i>Polyangium</i>	Roots	<i>Gentiana siphonantha</i>	[30]*
(+)-Pulegone, (-)-menthone	<i>Pseudomonas fluorescens</i> WCS417r	Leaves	<i>Mentha piperita</i> L. (Peppermint)	[36]
α -terpineol, Eugenol	<i>Bacillus subtilis</i> GB03	Leaves	<i>Ocimum basilicum</i> L. (Sweet basil)	[37]
Sesquiterpene				
Germacrene D	Bacteria pools isolated from roots	Roots	<i>Echinacea purpurea</i> (L.) Moench	[97]
β -caryophyllene				
Diterpene				
Steviol glycosides	<i>Bacillus safensis</i> STJP	Leaves	<i>Stevia rebaudiana</i>	[41]
Steviol glycosides	<i>Enterobacter hormaechei</i> H2A3 and H5A2	Leaves	<i>Stevia rebaudiana</i>	[42]
Andrographolide	<i>Micrococcus luteus</i> ASd6	Leaves	<i>Andrographis paniculata</i> (Burm. F) Nees	[46]
Tanshinone	<i>Cladosporium tenuissimum</i> DF11 (fungus)	Roots	<i>Salvia miltiorrhiza</i>	[49]
Tanshinone	<i>Pseudomonas brassicacearum</i> sub sp. <i>neaurantiaca</i>	Roots	<i>Salvia miltiorrhiza</i>	[50]
Triterpene and tetraterpene				
Glycyrrhizic acid	<i>Rhodoplanes</i>	Roots	<i>Glycyrrhiza uralensis</i> Fisch	[52]*
Glycyrrhizic acid	<i>Bacillus amyloliquefaciens</i> FZB42,	Roots	<i>Glycyrrhiza uralensis</i> Fisch	[55]
Astragaloside I, II, III	<i>Stenotrophomonas</i>	Roots	<i>Astragalus mongholicus</i>	[58]*
Astragaloside IV	Bacteria consortium	Roots	<i>Astragalus mongholicus</i>	[59]
Ginsenoside	<i>Paenibacillus polymyxa</i>	Roots	<i>Panax ginseng</i> C.A. Meyer (Ginseng)	[61]
Ginsenoside	<i>Bacillus</i> sp. LB5–3	Roots	<i>Panax ginseng</i> C.A. Meyer (Ginseng)	[62]
Crocin, safranal	<i>Bacillus mojavensis</i> CS4EB32 <i>Burkholderia gladioli</i> E39CS3	Flowers	<i>Crocus sativus</i> L. (Saffron crocus)	[65]
Polyphenols				
Liquiritin	<i>Rhodoplanes</i>	Roots	<i>Glycyrrhiza uralensis</i> Fisch	[52]*
Liquiritin	<i>Bacillus amyloliquefaciens</i> FZB42	Roots	<i>Glycyrrhiza uralensis</i> Fisch	[55]
Calycosin-7-glucoside	<i>Phyllobacterium Inquilinus</i>	Roots	<i>Astragalus mongholicus</i>	[59]*
Kaempferol-7-O-glucoside	<i>Ruminococcaceae bacterium</i> GD7	Roots	<i>Paeonia lactiflora</i> Pall	[71]*
Kaempferol3,7-di-O-glucoside				
Flavonoids	<i>Ruminococcaceae</i>	Leaves	<i>Ginkgo biloba</i>	[73]*
Piceatannol, kyotorphin	<i>Burkholderia ambifaria</i> LK-P4	Leaves	<i>Anoectochilus roxburghii</i>	[75]

(continued on next page)

Table 1 (continued)

Metabolites	Microbes	Plant tissue	Plant species	Reference
Flavonoids, total polyphenols	Bacteria consortium	Flowers	<i>Tagetes patula</i> L. (French marigold)	[76]
Alkannin/shikonin	<i>Labrys Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium Penicillium</i> (fungus)	Roots	<i>Alkanna tinctoria</i> L.	[77]*
Alkannin/shikonin	<i>Chitinophaga</i> sp. <i>Allorhizobium</i> sp. <i>Duganella</i> sp. <i>Micromonospora</i> sp.	Roots	<i>Alkanna tinctoria</i> L.	[78]
Alkannin/shikonin and their derivatives	<i>Chitinophaga</i> sp. R-73072 <i>Xanthomonas</i> sp. R-73098 <i>Pseudomonas</i> sp. R-71838	Roots	<i>Lithospermum officinale</i> L.	[79,80]
Alkaloids				
Matrine and Oxymatrine	<i>Actinobacteria phylum</i>	Roots	<i>Sophora flavescens</i>	[86]*
Aconitine	<i>Brevundimonas</i> (bacteria) <i>Cladosporium</i> (fungus)	Roots	<i>Aconitum vilmorinianum</i> Komarov	[87]*
Pyrrroloquinoline alkaloids	<i>Azotobacter chroococcum</i> <i>Pseudomonas putida</i>	Roots	<i>Adhatoda vasica</i> Nees	[89]
Vinblastine and vincristine	<i>Pseudomonas fluorescens</i> 169 <i>Azospirillum brasilense</i> Ab-101	Roots	<i>Catharanthus roseus</i> L.	[90]

Although it is unclear whether the correlations reflect the microbes' chemotrophic preference to these plant metabolites, such a possibility does not exclude the other possibility that the correlations resulted from microbe-enhanced production of the monoterpenes. Both possibilities remain to be examined.

In a study of the medicinal plant *Origanum vulgare* L. ssp. *vulgare* and its endophytes, the bacterial volatile organic compounds (VOCs) were found to contain monoterpenes, such as α -pinene and 3-carene that were also produced by the host plant [32,33]. Nonetheless, microbial emission VOCs can be important mediators of the microbe-enhanced plant production of monoterpenes, as exemplified in the studies of peppermint (*Mentha piperita* L.) and sweet basil (*Ocimum basilicum* L.). Peppermint is a medicinal herb traditionally used for treatments of fever, cold, digestive, anti-viral, anti-fungal and oral mucosa and throat inflammation [34]. Essential oils are among the major secondary metabolites responsible for the aroma effects of peppermint. The peppermint essential oils, which mainly consist of the monoterpenes menthol, menthone, neomenthol and iso-menthone, possess pharmacological effects such as anti-inflammatory, antimicrobial, immunomodulatory, and neuroprotective activities [35]. The VOCs emitted from a plant-beneficial *Pseudomonas fluorescens* strain was found to cause a 2-fold increase in the production of peppermint essential oils, among which the two major components, (+)-pulegone and (-)-menthone, showed increased biosynthesis in the *P. fluorescens*-treated plants [36]. Similarly, VOCs from a plant-beneficial *Bacillus subtilis* strain increased the two major essential oil components from the terpene-rich sweet basil (*Ocimum basilicum* L.), α -terpineol and eugenol, by approximately 2- and 10-fold, respectively [37]. A recent study attributed the microbe-enhanced production of peppermint essential oils to the increased levels of the phytohormones jasmonic acid (JA) and salicylic acid (SA), as well as a higher density of glandular trichomes where the

essential oils were produced [38]. Thus, it is an interesting question how certain microbial VOCs may trigger these *in planta* processes that lead to the enhanced production of monoterpenes.

2.2. Diterpenes

Steviol glycosides are a group of diterpene glycosides naturally present in only a few plant species, most notably *Stevia rebaudiana* [39,40]. These plant metabolites possess various medicinal activities including antidiabetic, antihypertensive, anti-inflammatory, antioxidant, anticancer, and antidiarrheal activities, in addition to exhibiting a superior sweetener proficiency than sucrose and are noncaloric, non-cariogenic, and nonfermentative [39,40]. Certain bacteria strains isolated from either the rhizosphere or the endosphere of *S. rebaudiana* were recently shown to increase steviol glycosides contents in their host plants. *Bacillus safensis* STJP, a biocontrol strain isolated from the *S. rebaudiana* rhizosphere, suppressed the fungal pathogen *Alternaria alternata* and increased steviol glycosides contents in *S. rebaudiana* infected by *A. alternata*, especially when used in combination with the mycorrhiza *Glomus fasciculatum* ABTEC [41]. Treatments of *S. rebaudiana* with *Enterobacter hormaechei* strains H2A3 and H5A2, which were isolated from the *S. rebaudiana* endosphere, resulted in increased steviol glycosides contents and the transcript upregulation of the steviol glycosides biosynthesis genes including *KO*, *KAH*, *UGT74G1*, and *UGT76G1* [42]. Interestingly, bacterial synthesis of rebaudioside A was observed for a strain of *Pantoea vagans* that was endophytic to *S. rebaudiana* seeds [43]. Thus, the microbe-increased accumulation of steviol glycosides in the *S. rebaudiana* plants might also be contributed directly by the presence of the microbes. On the other hand, microbial consumption of the plant-produced terpenoids may exist, as implied by the correlation between the expression levels of terpene synthesis genes in *A. macrocephala* rhizome and the abundance of the rhizome bacterial genes that are functionally related to terpenoid metabolism [44,45].

Andrographolide from *Andrographis paniculata* (Burm. F) Nees is a diterpenoid lactone widely used in Indian medicine for snake bite and for the treatment of hepatitis. The *Micrococcus luteus* strain ASd6, which was isolated as an endophyte from *A. paniculata*, was shown to substantially enhance the andrographolide content in *A. paniculata* plants [46]. Tanshinones, a class of diterpenes synthesized and accumulate in the root periderm of *Salvia miltiorrhiza*, possess various pharmacological effects including anti-tumor properties [47,48]. The endophytic fungus *Cladosporium tenuissimum* DF11, which was isolated from *S. miltiorrhiza* roots, was found to increase tanshinone levels via gene upregulation of a group of key enzymes in the tanshinone biosynthesis pathway [49]. Similarly, several *S. miltiorrhiza* endophytic bacteria strains significantly improved the activities of 3-hydroxy-3-methylglutaryl-1-CoA reductase and 1-deoxy-D-xylulose-5-phosphate synthase, which are two enzymes important for tanshinone biosynthesis, resulting in increases in tanshinone contents in the hairy roots [50]. In particular, *S. miltiorrhiza* treated with *Pseudomonas brassicacearum* sub sp. *neaurantiaca* displayed increased dihydrotanshinone I and cryptotanshinone contents by 19-fold and 11-fold, respectively, while the total tanshinone contents increased 3.7-fold compared to the control. However, it should be noted that all the endophytic bacteria treatments also caused up to 55 % reductions in the biomass of *S. miltiorrhiza* root hairs [50].

2.3. Triterpenes and tetraterpenes

The medicinal plant *Glycyrrhiza uralensis* Fisch is widely used to treat multiple diseases, and its efficacy is largely attributed to liquiritin and glycyrrhizic acid [51,52]. Glycyrrhizic acid is a triterpenoid glycoside with multiple anti-cancer-related pharmacological activities [53], while liquiritin is a flavonoid that can be utilized to prevent inflammation and relieve pain [54]. An interesting phenomenon about *G. uralensis* is that wild plants produced significantly more liquiritin and glycyrrhizic acid than cultivated plants [51,52]. Comparative multi-omics analyses

between the wild and cultivated *G. uralensis* revealed that the bacteria genus *Lysobacter* was remarkably more abundant in the cultivated plant rhizosphere than the wild plant rhizosphere, and that the expression of some key genes involved in liquiritin and glycyrrhizic acid biosynthesis were strongly negatively correlated with the abundance of *Lysobacter* [51,52]. In addition, the bacteria genus *Rhodoplanes* was found to be positively correlated with the accumulation of both liquiritin and glycyrrhizic acid, as well as be more abundant in the rhizosphere of wild *G. uralensis* compared to cultivated plants [51,52]. It remains unclear whether the chemical properties of the soil were different for the wild and cultivated *G. uralensis* plants, therefore, while the correlative observations suggest that the rhizosphere microbiome is important for the production of liquiritin and glycyrrhizic acid in *G. uralensis*, such an inference may be confirmed by treating *G. uralensis* with the isolated bacteria strains. In another study, bacterial influences on the contents of liquiritin and glycyrrhizic acid were observed with a *Bacillus amyloliquefaciens* strain, which alleviates the drought stress-induced reduction of liquiritin and glycyrrhizic acid contents in *G. uralensis* [55]. The alleviation was proposed to be mediated through the concomitantly increased JA accumulation in the bacteria-treated plants [55], but the hypothesis remains to be examined.

Bacteria-enhanced accumulation were also observed in two other groups of triterpene glycosides, namely astragalosides from *Astragalus mongholicus* and ginsenosides from *Panax ginseng* C. A. Meyer. The dried roots of *A. mongholicus* is a traditional Chinese herbal medicine used for immune stimulant, tonic, antioxidant, hepatoprotectant, diuretic, anti-diabetic, anticancer, and expectorant effects [56]. The aqueous extract of *A. mongholicus* roots contains astragalosides as the major components with anti-cancer activities [57]. In a recent study, the contents of astragaloside I (Ast I), AstII, and AstIII were found to be positively correlated with the abundance of *Stenotrophomonas* in the *A. mongholicus* rhizosphere [58]. Microbial enhancement of astragaloside contents was directly demonstrated in *A. mongholicus* inoculated with a bacteria consortium, which increased the contents of astragaloside IV by 183.83 % and 164.97 % under moderate and severe drought stress, respectively [59]. Ginseng (*Panax ginseng* C. A. Meyer) is known for its ginsenosides used for the prevention and treatments of cardiovascular and cerebrovascular diseases as well as for other pharmacological effects [60]. Inoculation of soil-grown ginseng plants with the foliar endophyte *Paenibacillus polymyxa* by means of foliar application combined with irrigation enhanced the plant concentration of ginsenosides [61]. Similarly, another ginseng foliar endophyte named LB 5-3 simultaneously increased biomass and ginsenoside accumulation in adventitious ginseng root culture [62]. These dual benefits are in contrast to the effects caused by exogenous application of the phytohormone JA, which reduced ginseng biomass in the root culture although it increased ginsenoside accumulation [63].

The tetraterpene compound crocin is one of the major apocarotenoids in the medicinal plant *Crocus sativus* L., of which the therapeutic properties are mostly attributed to its unique apocarotenoids with strong free radical scavenging activity [64]. A recent study of *C. sativus* endophytes showed that the plants were preferentially colonized by the genus *Bacillus*, followed by *Burkholderia* and *Pantoea* [65]. Two strains of the isolated endophytes, *Bacillus mojavensis* CS4EB32 and *Burkholderia gladioli* E39CS3, were shown to increase the expression of apocarotenoid biosynthetic genes in *C. sativus* [65], such as the phytoene synthase that carries out the first step in the carotenoid biosynthesis [66], and the beta-carotene hydroxylase that catalyzes β -carotene hydroxylation and generates the precursor of apocarotenoids [67]. Consistent with the gene induction, the endophyte treatments resulted in a marked increase in the concentration of apocarotenoids, including the tetraterpene crocin and safranal that is a monoterpene aldehyde [65].

3. Microbe-increased production of polyphenols

Polyphenols are phenolic compounds ranging from simple phenolic

molecules to highly polymerized compounds [68]. These structurally diverse compounds are commonly subclassified into flavonoids, which have a structure based on or similar to that of flavone, and non-flavonoid polyphenols such as phenolic acids [69]. Polyphenols display a variety of health-promoting effects, such as antioxidant, antibacterial, cardio-protective, and anti-inflammatory [70].

Recent studies of polyphenols in medicinal plants have highlighted the roles of plant-beneficial microbes. In the two *Paeonia lactiflora* Pall cultivars Zifengyu and Hangbaishao, the flavonoids and phenolic acid contents were significantly higher in the former than the latter [71], and the differences were attributed to the root endophyte *Ruminococcaceae* bacterium GD7 that was significantly correlated with the levels of phenolic acid and flavonoids, including kaempferol-7-O-glucoside and kaempferol-3,7-di-O-glucoside [71], which possess antiproliferation effects on cancer cells [72]. Similarly, the flavonoid accumulation showed a positive correlation with the endophyte *Ruminococcaceae* in *Ginkgo biloba* [73]. In *A. mongholicus*, the bacteria genera *Phyllobacterium* and *Inquilinus* in the endosphere were positively correlated with the content of calycosin [58], a flavonoid that has therapeutic effects on a variety of diseases [74]. In *Anoectochilus roxburghii* that produces kyotorphin and piceatannol, the bacteria treatment with *Burkholderia ambifaria* LK-P4 increased the plant contents of these two phenolic compounds, which can significantly decrease blood glucose levels and protect the organs of mice with similar effects of glibenclamide tablet, a standard drug for the symptomatic treatment of diabetic mice [75]. In French marigold (*Tagetes patula* L.) that is used for skin and digestive conditions, the treatment of an endophytic bacteria consortium resulted in 56 % and 38 % in the contents of flavonoids and total polyphenols, respectively [76].

The roots of *Alkanna tinctoria* L. are rich in alkannin and shikonin, which are two enantiomeric polyphenols that induce excellent cytotoxicity in cancer cells [77]. A recent study of *A. tinctoria* grown in a greenhouse revealed that the plant developmental stage influenced both the metabolite contents and the plant root microbial diversity, and that the bacterial genera *Labrys* and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* as well as four species of the fungal genus *Penicillium* were positively correlated with higher contents of alkannin and shikonin [77]. Four bacteria strains from the root microbiome of wild *A. tinctoria*, including *Chitinophaga* sp., *Allorhizobium* sp., *Duganella* sp., and *Micromonospora* sp., were shown to significantly increase the contents of alkannin and shikonin in hairy roots culture of the same plant species [78]. In *Lithospermum officinale* L., the contents of alkannin and shikonin were significantly increased by treatments with either one of the three bacteria strains belonging to *Chitinophaga*, *Xanthomonas* or *Pseudomonas* [79]. Subsequent transcriptome analysis using the *Chitinophaga* strain revealed that the bacterium increased the expression levels of genes that appeared to be mainly involved in plant defense and in the biosynthesis of PSMs including alkannin and shikonin derivatives [80], implying a potential correlation between the bacteria-induced plant defense and the enhanced alkannin and shikonin accumulation in *L. officinale*.

4. Microbe-increased production of alkaloids

Alkaloids are naturally occurring toxic amines primarily found in plants, where they mainly function in defense against pathogens and herbivores. Based on their heterocyclic rings and biosynthetic precursors, alkaloids are classified into diverse categories including indole, purine, quinoline, isoquinoline, tropane, imidazole, among others [81, 82]. As medicinal compounds, alkaloids are particularly well known as anaesthetics, cardioprotective, and anti-inflammatory agents [83].

Matrine and oxymatrine function as the most active alkaloids in *Sophora flavescens* for the pharmacological effects on cancers, viral hepatitis, and cardiac diseases [84,85]. A recent study analyzed the association between rhizosphere microbiome and alkaloids accumulation in *S. flavescens* with different geographic locations and plant ages. The bioinformatics results indicated that some taxa belonging to

Actinobacteria and *Chloroflexi* were potential stimulants for matrine and oxymatrine accumulation, whereas certain bacteria from the phyla *Gemmatimonadetes* and *Acidobacteria* showed negative correlation with the accumulation of matrine and oxymatrine [86]. Similarly, in *Aconitum vilmorinianum* Komarov that is used for treating rheumatism and pains, the bacteria *Brevundimonas* and fungus *Cladosporium* in root microbiome were suggested to be positively correlated with the presence of aconitine [87], which is an alkaloid with multiple pharmacological effects such as analgesic and anti-inflammatory [88].

Microbe-enhanced alkaloid accumulation in medicinal plants were recently observed in *Adhatoda vasica* Nees, which is used in the treatments of chronic bronchitis and diarrhea, among other diseases [89], and in *Catharanthus roseus* L. that is the sole resource of vinblastine and vincristine, two alkaloids with powerful anticancer activities [90]. By using hairy root developed from the cut edges of *A. vasica* leaf discs, multiple *Azotobacter chroococcum* and *Pseudomonas putida* strains were shown to increase the accumulation of pyrroloquinazoline alkaloids, including vasicine, vasicinone, vasicine acetate, 2-acetyl benzyl amine, vasicinolone, deoxyvasicine and vasicol [89], which are the most bioactive phytochemicals in *A. vasica* with anti-inflammatory and antimicrobial activities [91]. The bacteria-enhanced production of pyrroloquinazoline alkaloids was attributed to the increased activity of anthranilate synthase [89], which functions as a rate-limiting factor for the biosynthesis of pyrroloquinazoline alkaloids [92]. In *C. roseus* plants, treatments with either *P. fluorescens* strains 169 or *A. brasilense* strains Ab-101 increased the expression of genes involved in the biosynthesis of vinblastine and vincristine, resulting in significant increases in the contents of these two terpenoid indole alkaloids (TIAs) [90]. Interestingly, the TIA biosynthesis pathway in *C. roseus* was shown to be induced by SA, methyl-JA, and ethylene [93,94], which are phytohormones known to mediate plant responses to microbes.

5. Conclusions and perspectives

Plants present a huge reservoir of secondary metabolites for medicinal usages. Many open-access databases of pharmacological plant metabolites are available, such as the 25 medicinal herbs databases recently reviewed in Fathifar et al. (2023) [95] and PubChem (<https://pubchem.ncbi.nlm.nih.gov>) that is the world's largest collection of freely accessible chemical information. Due to the technical difficulties in de novo chemical synthesis, extraction from plants remains the most economic strategy for most of the complex PSMs, in addition to using the medicinal plants directly. Plant-beneficial microbes offer effective tools for increasing the contents of pharmacological PSMs in medicinal plants. Under such a specifically defined scope, the original studies reviewed herein account for only a small portion of the vast literature of microbial influences on plant secondary metabolites; nonetheless, these reports sufficiently demonstrated the increasing interests in exploiting

microbial capacities for enhancing plant production of pharmacological metabolites. This research field has recently been boosted by the advances in microbiome research techniques, and is emerging as a new frontier of medicinal plant research, where a number of key questions beyond the beneficial effects await for elucidation (Fig. 1).

Currently some examples of the beneficial effects were proposed based on bioinformatics analyses that indicated positive correlations between the plant microbiome members and the PSMs of interest. Both the plants and their microbiome are subjected to the influences by the environment. Hence, it is helpful or even necessary to take environmental factors into account when considering the correlation between a medicinal PSM and the plant microbiome, especially for medicinal plants that display geo-herbalism. Plants provide not only physical niches but also nutrient molecules to microbes, thus a positive correlation between a microbe and a PSM may reflect the microbe's chemotrophic preference to the PSM, in addition to the possibility that an increased PSM level results from an increased abundance of the microbe, although the two possibilities are not mutually exclusive. This ambiguity is related to another interesting question as how the microbe may benefit from the increased accumulation of the correlated PSM. In addition, positive correlations between a PSM and an endophyte may be due to the microbial capacity of producing metabolites that are similar or identical to the PSM of interest. For instance, dozens of fungal strains have been identified as taxol-producing endophytes from *Taxus* spp [96]. In the cases where the microbe-increased PSMs carry antimicrobial functions, it is intriguing whether the microbes are resistant to those PSMs.

Increasing the accumulation of PSMs that carry defense functions is a common response to biotic stimuli including beneficial microbes. Although the microbe-increased PSM accumulation may be mediated through the phytohormones JA, SA, and ethylene, which play important roles in plant defense, treating the medicinal plants with beneficial microbes likely would be superior to exogenous applications of the phytohormones, because the mutualism with beneficial microbes improves the plant's growth and/or stress-resistance, whereas boosting the plant defense alone usually leads to retarded plant growth due to a trade-off balance between growth and defense. In the cases where the microbe-enhanced PSM accumulation was mediated through the plant defense, it is an important question about how the defensive effect on metabolism was specifically triggered by certain microbes instead of the other microbes. While examples of microbe-enhanced pharmacological PSMs in medicinal plants are increasing, clear insights into the underlying molecular mechanism are commonly hindered by a lag in the knowledge of functional genomics of these plant species. Meanwhile, the microbial factors that trigger the specific metabolic responses are crucial for understanding the mechanisms, and therefore should receive more attentions. Another important question regarding the mechanisms is how specific the microbial effects on the pharmacological PSMs are, in

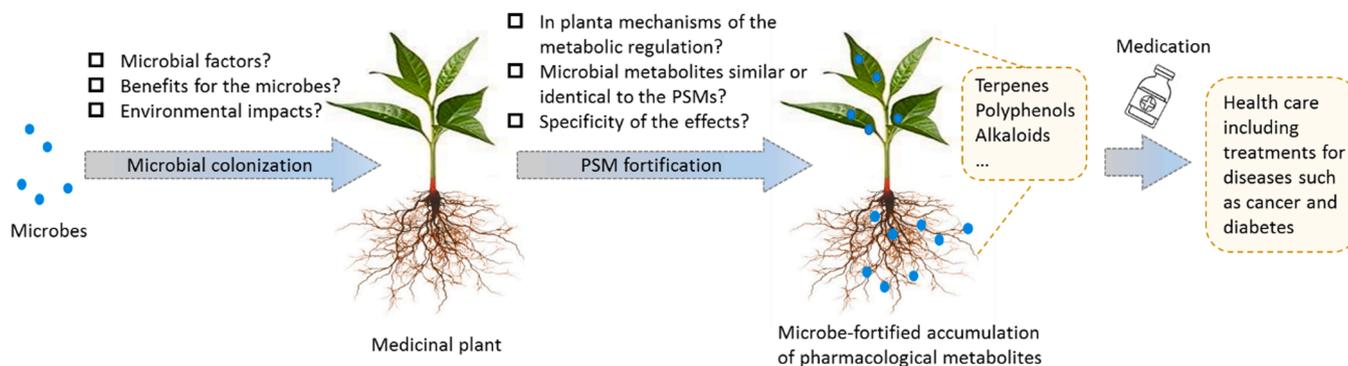


Fig. 1. A schematic model for microbial fortification of pharmacological metabolites in medicinal plants. Emerging evidence has highlighted the effectiveness of microbes, either plant epiphytic or endophytic, in increasing the contents of pharmacological metabolites in medicinal plants, albeit a number of important questions remain to be addressed.

other words, whether the microbes alter the metabolism of other PSMs, particularly those that may negatively affect the pharmacological quality of the medicinal plants. Explicit molecular insights into the underlying mechanisms will underpin microbial applications for metabolic fortification in medicinal plants.

CRedit authorship contribution statement

X.W. and Y.Y. researched and wrote a draft. H.Z. researched and wrote the article. All authors reviewed and/or edited the article before submission. The authors read and approved the final manuscript.

Declaration of Competing Interest

Authors declare that they have no competing interests.

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