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REVIEW ARTICLE

# Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts

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**Summary** Medicinal plants have been used for thousands of years in folk medicines and still are used for their health benefits. In our days medicinal plants are exploited for the isolation of plant-derived drugs as they are very effective and have relatively less or no side effects. However, the natural resources of medicinal plants are gradually exhausted and access to plant bioactive compounds is challenged by the low levels at which these products accumulate in native medicinal plants. For instance, to meet the market demands of 3 Kg per year of vinca alkaloids, powerful plant-derived anticancer drugs, $1.5x10^6$ Kg dry leaves are required. In this regard, this review aims to highlight the fact that endophytic fungi residing in medicinal plants are capable to biosynthesize pharmacologically active secondary metabolites similar or identical to those produced by their host medicinal plant. Furthermore, the evolutionary origin of the genes involved in these metabolic pathways as well as the approaches designed to enhance the production of these metabolites by the isolated endophytic fungi are also discussed.

*Additional key words:* metabolites from endophytic bacteria and actinomycetes, chemical ecology

## Introduction

Plant endophytes consist of bacterial and fungal communities that colonize and spend the whole or part of their life cycle inside the plant tissues, without instigating any noticeable symptoms of infection or visible manifestation of disease to their hosts (Petrini and Fisher, 1990). Evidence of plant-associated microorganisms found in the fossilized tissues of land plants stems and leaves suggests that endophyte–plant associations may have evolved along with the evolution of higher land plants (Krings *et al*., 2007). Nearly all vascular plant species studied were found to harbor endophytic bacteria and/or fungi (Rodriguez *et al*., 2009; Hardoim *et al*., 2015). They are found to be present in virtually all organs of a given plant host, and some are seed borne. Endophytes often confer considerable benefits to the host plant they inhabit, since they can promote the growth of host plants, enhance resistance to biotic and abiotic stresses (Rodriguez *et al*., 2009; Hardoim *et al*., 2015), and accumulate bioactive secondary metabolites (Kusari *et al*., 2012). The ecological role of secondary metabolites produced by endophytes is not clear. However, recent studies have shown that these metabolites are involved in deterrence of herbivory (Pannaccione *et al*., 2014), protection against fungal (Soliman *et al*., 2015) or bacterial pathogens (Mousa *et al*., 2017) and amelioration of plant abiotic stress (Hamayum *et al*., 2016).

## Bioactive secondary metabolites derived from medicinal plants are gradually decreasing - Alternative approaches for their production

Medicinal plants, as a rich source of nat-

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ural products, have been used to treat various ailments and have been the foundation for discovery and development of modern therapeutics (Pan *et al*., 2013). Up to 80 % of people in developing countries are totally dependent on herbal drugs for their primary healthcare. More than 51% of small molecule drugs approved between 1981 and 2014 were based on natural products, the rest being synthetic (Chen *et al*., 2016). With the increasing demand for herbal drugs, natural health products and secondary metabolites, the use of medicinal plants is growing rapidly throughout the world (Chen *et al*., 2016). However, we are facing the accelerated loss of wild medicinal plant species; one third of the estimated 50.000-80.000 medicinal plant species are threatened with extinction from overharvesting and natural anthropogenic habitat destruction (Chen *et al*., 2016). Furthermore, the feasibility of access to plant bioactive compounds is challenged by the low levels at which these products accumulate in native medicinal plants, the long growth periods required for plant maturation, and the difficulty in their recovery from other plant-derived metabolites (Staniek *et al*., 2014). For example, the taxol concentration is about 0.001–0.05% in *Taxus brevifolia*, which is the most productive species. Thus, 15 kg of Taxus bark, three trees, are required for production of 1 g, while every cancer patient requires about 2.5 g (Malik *et al*., 2011).

Therefore, it is important to find alternative approaches to produce the medicinal plant-derived biologically active compounds, in particularly those derived from endangered or difficult-to-cultivate plant species, to meet the medical demand. This can be achieved by the application of plant cell and tissue culture, heterologous production, total chemical synthesis, semi-synthesis, or by starting with a microbially - produced or plant-extracted natural product occurring more abundantly in nature (Atanasof *et al*., 2015; Rai *et al*., 2016; Ramirez-Estrada *et al*., 2016) or by exploiting the ability of endophytic fungi residing in plants to produce the same or similar bioactive compounds as their hosts (Zhao *et al*., 2011). In this review, we aim to show that the large number of medicinal plants used for the isolation of medically important bioactive compounds harbor endophytic fungi capable of host-independent biosynthesis of the same or similar bioactive secondary metabolites as their hosts. This review will also discuss the evolution and origin of pathways involved in the biosynthesis of these bioactive compounds and potential approaches aiming to enhance their production.

## Medicinal plants harbor endophytic fungi capable of mimicking their host plant secondary metabolite profile- Case studies on medicinal plants producing metabolites of known medical importance

Since the first report of endophyte *Taxomyces andreanae* that produces the same bioactive secondary metabolite taxol (paclitaxel) as its host *Taxus brevifolia* in 1993 (Stierle *et al*., 1993), several studies have shown that plant-derived secondary metabolites are produced by endophytes (Zhao *et al*., 2011). In this section, we will present a literature survey aiming to show that medicinal plants used for isolation of medically important secondary metabolites usually harbor endophytic fungi which are capable of host-independent biosynthesis of these metabolites. In each one of the presented case studies, emphasis will be placed on the plant species, the organ where the bioactive compound is accumulated and the organ from which the active compound-producing fungi were isolated.

### *Salvia* sp. (Lamiaceae)

*Salvia* species have many important medicinal properties with proven pharmacological potential. Some of these properties may be mediated by biologically active polyphenols or terpenoids (Wu *et al*., 2012). Two kinds of bioactive compounds, tanshinones (tanshinone I, tanshinone IIA, tanshinone IIB, isotanshinone I, and cryptotanshinone) and salvianolic acids (salvianolic acid

and rosmarinic acid) have been found in the roots and leaves of *S. miltiorrhiza*, respectively. Tanshinones belong to diterpenoid quinones, and are considered as potent anti-carcinogenic, antiatherosclerosis, and antihypertensive, whereas salvianolic acids are phenolic acids, which are mainly responsible for beneficial effects on cardiovascular and cerebrovascular diseases (Chun-Yan *et al*., 2015). Several *Salvia* species produce the bioactive phenolic labdane-type diterpenes rosemarinic acid, carnosic acid and carnosol. These compounds show distinct anti-oxidant activity with carnosic acid carnosol being approved food additives (Wu *et al*., 2012). *Salvia divinorum* produces a novel diterpenoid, salvinorin A, which is a powerful hallucinogen in humans and shows a selective, high efficacy agonist activity (Butelman and Kreek, 2015).

Eighteen endophytic fungal strains have been isolated from the roots of *Salvia miltiorrhizae,* the site of tanshinones accumulation, and 58 fungal strains from the leaves, the main site of salvianolic acid accumulation. Liquid culture extracts of all the fungi were screened for the presence of tanshinones or salvianolic acid, respectively. One fungus in each case was proven to produce tanshinones or salvianolic acid compared with authentic standards. However, the yield was quite low; about 4µg/L for tanshinones and 47µg/L for salvianolic acid (Ming *et al*., 2013; Li *et al*., 2016).

### *Catharanthus roseus* (L.) G.Don (Apocynaceae)

*Catharanthus roseus* is well known for the production of several anticancer vinca alkaloids such as vincristine, vindesine, vinorelbine, vinblastin and the recently discovered vinflunine (Kumar *et al*., 2014). The two major anticancer vinca alkaloids, vincristine and vinblastine, used in chemotherapy regimens, have been isolated from leaves (Kumar *et al*., 2014).

The different *C. roseus* plant organs harbour a plethora of endophytic fungi (Kharwar *et al*., 2008; Kumar *et al*., 2013; Palem *et al*., 2015; Kuriakose *et al*., 2016). Screening all the endophytes for the production of vinca alkaloids revealed that only endophytic fungi residing in the leaves of *C. roseus* were capable of producing vinblastine and vincristine. These endophytic fungi were identified as *Fusarium oxysporum, Talaromyces radicus* and *Eutypella* spp. The drugs were purified by TLC and HPLC and authenticated using UV-Vis spectroscopy, ESI-MS, MS/MS and $^{1}$H NMR. Culture filtrates of the fungi yielded >55 µg/L of vinblastine or vincristine, respectively (Kumar *et al*., 2013; Palem *et al*., 2015; Kuriakose *et al*., 2016).

### *Coleus forskohlii* (Willd.) Briq. (Lamiaceae)

*Coleus forskohlii* or Indian *Coleus* is a tropical perennial shrub of the *Lamiaceae* family and grows in the subtropical temperate climates of South-east Asia and India. The plant is extensively cultivated in southern India and the roots are used in Indian folk medicine for treating a broad range of human health disorders (Kavitha *et al*., 2010). The roots of the herb contain a pharmacologically active compound called forskolin that accumulates in the root cork (Pateraki *et al*., 2014). The approved and potential applications of forskolin range from alleviation of glaucoma, anti-HIV or antitumor activities, treatment of hypertension and heart failure to lipolysis and body weight control (Pateraki *et al*., 2017).

Screening of endophytic fungi isolated from inner tissues of root and stems of *C. forskohlii* for the production of forskolin revealed that one of the endophytic fungi identified as *Rhizoctonia bataticola* was able to stably synthesize forskolin and interestingly, release it into the broth (Mir *et al*., 2015).

### *Macleaya cordata* (Willd.) R.Br. (Papaveraceae)

Sanguinarine (SA) is a benzophenanthridine alkaloid isolated from *Macleaya cordata* leaves, and is known to have a wide spectrum of biological activities, such as antibacterial, antihelmintic, antitumor and anti-inflammatory (Wang *et al*., 2014). SA is

used in feed additives for livestock production (Kantas *et al*., 2014). Most of the SA currently used in herbal supplements and medicines is extracted from *M. cordata*. Recently, SA has gained increasing attention as a potential agent in the treatment of cancer (Yu *et al*., 2014).

Screening of endophytic fungi isolated from leaves of *M. cordata* revealed that one of 55 isolates has the capacity to produce SA (Wang *et al*., 2014).

### *Cajanus cajan* (L.) Millsp. (Fabaceae)

*Cajanus cajan* (pigeon pea) is a grain legume crop in semitropical and tropical areas of the world. The extract of pigeon pea leaves exhibit therapeutic effects on sickle cell anemia, plasmodiosis, and hepatic disorders. Moreover, pigeon pea roots are used as a sedative, a vulnerary preparation. The active constituents of pigeon pea are flavonoids and stilbenes. Cajaninstilbene acid (CSA) is one of the major stilbenes found in pigeon pea. Pharmacological studies have revealed that CSA exhibited anti-inflammatory and analgesic effects. In addition, CSA has an antioxidant activity similar to that of the natural antioxidant resveratrol (Liang *et al*., 2013). Cajanol is a isoflavone isolated from pigeon pea roots. Pharmacological studies have shown that cajanol has antiplasmodial, antifungal and antimicrobial activities. In addition, cajanol has been described as a novel anticancer agent, which induced apoptosis in human breast cancer cells (Luo *et al*., 2011).

A total of 245 endophytic fungi isolated from roots, stems and leaves of pigeon pea plants were screened for the production of cajaninstilbene acid or cajanol. Three fungal strains isolated from leaves were capable of producing CSA and one strain isolated from roots stably produced cajanol at a concentration of 500μg/L (Zhao *et al*., 2012; Zhao *et al*., 2013).

### *Cephalotaxus hainanensis* H.L.Li (Cephalotaxaceae)

*Cephalotaxus hainanensis* H. L. Li is an indigenous conifer tree of China. The bark and leaves of *Cephalotaxus* have been used in Chinese folk medicine as anticancer agents, and its biological active constituents were proved to be alkaloids. Among these alkaloids, homoharringtonine (HHT) was shown effective against acute myeloid leukemia and has recently been approved by the Food and Drug Administration for the treatment of chronic myeloid leukemia (Hu *et al*., 2016).

A large number of endophytic fungi have been obtained from *Cephalotaxus* phloem. The bioactive compounds isolated from their culture extracts were characterized as sesquiterpenoids, anthraquinones and aromatic compounds, which exhibited cytotoxic and antibacterial activities (Lu et al., 2012; Xue *et al*. 2012; Zheng *et al*., 2011). The hunt for an HHT-producing endophytic fungus was eventually successful following the screening of 213 strains isolated from the bark of *Cephalotaxus* trees grown in China and Thailand. The fungus was identified as *Alternaria tenuissima* and stably produced 100 μg/L HHT (Hu *et al*., 2016).

### *Cinchona* spp. (Rubiaceae)

The bark of the stem and roots of various trees of the genus *Cinchora* produce quinine alkaloids (quinine, quinidine, cinchonidine and cinchonine), which were the only effective treatment of malaria for more than four centuries. *Cinchona* bark and its alkaloids remained the most efficient treatment of malaria until the 1940s when chloroquine and other synthetic antimalarial compounds were developed (Kaufman and Ruveda, 2005). With the development of resistant malaria strains, the quest for new antimalarial compounds was successful with the discovery of artemisinin from a Chinese herbal medicine based on *Artemisia annua* L. (Tu, 2011).

Twenty-one endophytic fungi have been isolated from *Cinchona ledgeriana* young plant stems and screened for the presence of Cinchora alkaloids. These fungi comprised of *Phomopsis, Diaporthe*, *Schizophyllum*, *Penicillium*, *Fomitopsis* and *Arthrinium* species while fermentation studies demon-

strated that all produce quinine and quinidine, as well as cinchonidine and cinchonine (Maehara *et al*., 2011; Maehara *et al*., 2013).

***Passiflora incarnata* (Passifloraceae)**

Passiflora consists of 500 species that are found mostly in warm and tropical regions. *Passiflora incarnata* leaves were found to contain several active compounds, including alkaloids, phenols, glycosyl flavonoids, and cyanogenic compounds. The major compounds present in *P. incarnata* are C-glycosyl flavonoids (vitexin, isovitexin, orientin and chrysin) and b-carbolinic alkaloids (harman, harmin, harmalin, harmol, and harmalol). Among these natural products, chrysin has shown interesting biological activities, including antibacterial, anti-inflammatory, anti-diabetic, anxiolytic, hepatoprotective, anti-aging, anticonvulsant and anticancer effects (Seetharaman *et al*., 2017).

Three endophytic fungi identified as *Altenaria alternata*, *Colletotrichum capsici*, and *C. taiwanense* were isolated from leaves of *P. incarnata* and production of fungal chrysin was confirmed through UV-vis spectroscopy, FT-IR, LC-ESI-MS, and $^{1}H_{1}$ NMR analysis of their extracts. The quantitative HPLC analysis revealed that the yield of chrysin from *A. alternata* was higher when compared with previously reported bioresources (Seetharaman *et al*., 2017).

***Fritillaria cirrhosa* D.Don (Liliaceae)**

Bulbus *Fritillaria* have been used in traditional Chinese medicine for more than 2000 years, and at present, they are among the most widely used antitussive and expectorant drugs. The major biological active ingredients of Bulbus *Fritillaria cirrhosa* are steroidal alkaloids, such as peimisine, imperialine-3β-D-glucoside, and peimine (Wang *et al*., 2011).

Several dosens of endophytic fungi were isolated from fresh bulbus of *Fritillaria unibracteata* var. *wabensis*. One of these fungal endophytes, *Fusarium redolens* 6WBY3 was capable of producing and secreting in the culture medium peimisine and imperialine-3β-D-glucoside whereas a second endophytic fungus was found to secrete peimisine and peiminine. Interestingly, a large number of the remaining endophytes were able to produce large amounts of antioxidants, such rosemarinic acid (Pan *et al*., 2014; Pan *et al*., 2015; Pan *et al*., 2017).

***Huperzia serrata* (Thunb. ex Murray) Trevis (Huperziaceae)**

*Huperzia serrata* is a traditional Chinese herb medicine and has been extensively used for the treatment of a number of ailments, including contusions, strains, swellings, schizophrenia, myasthenia gavis and organophosphate poisoning. These pharmaceutical applications of *H. serrata* are mainly due to its biologically active lycopodium alkaloids. Among the lycopodium alkaloids, huperzine A (HupA) was found to possess potent acetylcholine esterase inhibition (AChEI) and is clinically used for the treatment of Alzheimer's disease (Zhao *et al*., 2013). The content of HupA in the leaf is richer than that in the stem and root of *H. serrata* (Gu *et al*., 2005).

Several groups have isolated endophytic fungi from leaves, stems and roots of *H. serrata*. Screening culture extracts of these fungi for HupA production revealed that most of HupA-producing fungi were isolated from leaf tissues (Su *et al*., 2017). The HupA-producing endophytic fungi belong to *Penicillium griseofulvum*, *Penicillium* sp., *Aspergillus flavus*, *Mycoleptodiscus terrestris*, *Trichoderma* sp., *Colletotrichum gloeosporioides* strain ES026 and *Shiraia* sp.. The productivity of these strains is less than 60-90 µg/L, with *Shiraia* sp. Slf14 being the best producer (327.8 µg/L) (Su *et al*., 2017). Interestingly, many *H. serrata* endophytic fungi with AChE inhibitory activity did not contain HupA in their extracts (Su *et al*., 2017; Wang *et al*., 2016) suggesting that some endophytic fungi produce new compounds with activity against AChE.

***Rhodiola* spp. (Crassulaceae)**

*Rhodiola rosea* is a perennial herbaceous plant that belongs to the family *Crassu-*

*laceae*. This species is mainly distributed in high altitudes of >2,000 m in the Arctic and mountainous regions throughout Asia and Europe. This typical alpine plant has been widely used as an important food crop and folk medicine since ancient times by many countries, such as Sweden, Russia, India, and China (Chiang *et al*., 2015). *Rhodiola* rhizome, as a traditional folk medicine, stimulates mental and physical endurance, counteracts depression, improves sleep quality, and prevents high-altitude sickness. Modern pharmacology research suggests that *Rhodiola* rhizome has received considerable attention because of its biological behavior, including antioxidant and anti-aging properties, anti-microwave radiation, antihypoxia and adaptogenic activities. Most of these effects are ascribed to phenolics, such as salidrosides and p-tyrosol, and glycosides like rosavins (Chiang *et al*., 2015).

Screening of 347 endophytic fungal strains isolated from rhizomes of *R. crenulata*, *R. angusta* and *R. sachalinensis* revealed that four endophytic fungi were capable of producing salidrosides and p-tyrosol (Cui *et al*., 2015). One of these endophytic fungi identified as *Phialocephala fortinii* was able to stably produce large amounts of salidrosides and p-tyrosol, 2.3 and 2 mg/ml of culture medium, respectively (Cui *et al*., 2016).

### *Solanum nigrum* L. (Solanaceae)

*Solanum nigrum* L., family *Solanaceae* is a well-known medicinal plant which possesses several biological activities such as antioxidant, hepato-protective, antiinflammatory, antipyretic, diuretic, antimicrobial and anticancer activities due to its flavonoid and steroidal alkaloids content (Jain *et al*., 2011). Solamargine, one of the major steroidal alkaloids in *S. nigrum* has been demonstrated to exhibit potent anticancer activity against colon, prostate, breast, hepatic and lung cancer cell lines (Jain *et al*., 2011). Solamargine is always found in a complex mixture with other glycoalkaloids such as solasonine and solanine, which makes solamargine isolation from the plant quite difficult (Milner *et al*., 2011). Chemical synthesis of solamargine is possible, however it does not appear to be practical as the overall yield was only 10.5%, requiring 13 steps (Wei *et al*., 2011).

Three fungal endophytes have been isolated from *S. nigrum* stems, leaves and fruits. Their culture extracts were screened for the potential production of steroidal alkaloids. The stem derived endophytic fungal strain *A. flavus* was able to steadily produce solamargine with a titer of about 250–300 µg/ L which is higher than the plant callus culture method (El-Hawary *et al*., 2016).

### *Piper longum* L. and *Piper nigrum* L. (Piperaceae)

Piperine is a major alkaloid present in the fruit of *Piper longum* and *Piper nigrum* and it is known to have a wide range of pharmaceutical properties including antibacterial, antifungal, hepato-protective, antipyretic, anti-inflammatory, anti-convulsant, insecticidal and antioxidant. The amount of piperine varies in plants belonging to the *Piperaceae* family; it constitutes 2% to 7.4% of both black pepper and white pepper (Corgani *et al*., 2017). Screening of endophytic fungi isolated from both plant species revealed the presence of piperine in culture extracts of endophytic *Periconia* strains isolated from leaves of *P. longum* (Verma *et al*., 2011) and *Colletotrichum gloeosporioides* from the stems of *P. nigrum* (Chithra *et al*., 2014).

### *Digitalis lanata* Ehrh. (Plantaginaceae)

Glycosides from plants of the genus *Digitalis* have been reported to be cardiotonic and are widely used in the treatment of various heart conditions namely atrial fibrillation, atrial flutter and heart failure. The bioactive glycosides accumulate in the leaves and to a less extent in other organs of the plant (Alonso *et al*., 2009).

A total of 35 fungal endophytes were isolated from stems and leaves, and screened for the production of secondary metabolites. Crude extracts of fungal cultures revealed the production of glycoside digoxin from cultures of five endophytic strains (Kaul *et al*., 2013).

***Capsicum annuum* L. (Solanaceae)**

Capsaicin, the pungent alkaloid of red pepper (*Capsicum annuum*), is present in large quantities in the placental tissue, the internal membranes and, to a lesser extent, the other fleshy parts of the fruits of *Capsicum*. The pharmacological properties of capsaicin include cardio protective influence, anti-lithogenic effect, anti-inflammatory and analgesia, thermogenic influence, and beneficial effects on gastrointestinal system (Srinivasan *et al*., 2016).

An endophytic fungal strain identified as *Alternaria alternata* has been isolated from fruits of *C. annuum* and has been found to produce and secrete capsaicin up to three generations (Devari *et al*., 2014).

***Ginkgo biloba* L. (Ginkoaceae)**

Ginkgo tree contains in bark and leaves flavones and terpenoide lactones, among which, bilobalide and ginkgolides (terpenoide lactones) have been shown to be beneficial to human health (Usai *et al*., 2011). Ginkgolide B has revealed potent antagonistic effects on platelet activating factors involved in the development of a number of renal cardiovascular, respiratory and central nervous system disorders (Usai *et al*., 2011) while bilobalide was found to exert neuroprotective effects (Kiewert *et al.,* 2008).

Screening of 27 endophytic fungal strains isolated from the bark of *G. biloba* trees revealed that only one isolate *F. oxysporum* SY0056, was capable of producing Ginkgolide B (Cui *et al*., 2012). The search for bilobalide -producing endophytic fungi was far more copious; a total of 57 fungal strains were isolated from stem, root, leaf, and bark of the plant *G. biloba* and their extracts were evaluated for the presence of bilobalide. Only the isolate *Pestalotiopsis uvicola* GZUYX13 residing in leaves was proven to be a bilobalide-producing fungus (Qian *et al*., 2016).

***Silybum marianum* (L.) Gaertn. (Asteraceae)**

Silymarin is a bioactive extract of the fruits of *Silybum marianum* and contains seven flavolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, and silydianin) with reported chemoprevention and hepatoprotective properties (Feher and Lengyel, 2012).

Twenty one endophytic fungi were isolated from stems, leaves, roots, and seeds of *S. marianum* and were examined for production of flavolignans (El-Elimat *et al*., 2014). Two of these compounds, silybin A and silybin B, have been extracted as fermentation products of two strains of *Aspergillus iizukae* isolated from the leaves and stems of *S. marianum,* respectively. Subcultur of one flavonolignan-producing strain revealed an attenuation of the production of flavonolignans. However, when autoclaved leaves of the host plant were added to the growth medium, the production of flavonolignans could be resumed (El-Elimat *et al*., 2014).

***Vinca minor* L. (Apocynaceae) and *Nerium indicum* Mill. (Apocynaceae)**

Vincamine indole alkaloids (vincamine, tabersonine and catharanthine) are widely found in plants of the *Apocynaceae* family and show beneficial properties for human, such as prevention of cerebrovascular, precaution of chronic ischemic stroke, and reduction of vascular dementia or memory impairment (Saurabh and Kishor, 2013). Vincamine is a precursor compound for other medicinal alkaloids such as 11-bromovincamine, ethyl-vincamine and vinpocetine, which have shown potential clinical therapeutic effect (Manda *et al*., 2015). Vincamine is accumulated in the leaves and stems of *Vinca minor* and *Nerium indicum*. Though abundant chemical synthesis and semi-synthesis research results have been reported, the main sources of vincamine indole alkaloids are stems and leaves of *Vinca minor* L.

Eleven fungal strains have been isolated from the stems and roots of *Nerium indicum* and fungal culture extracts were screened for the presence of indole alkaloids (Yin and Sun, 2011). One fungal strain, CH1, produces vincamine alkaloids as its host plant as determined by TLC, HPLC and LC–MS analysis. The yield of vincamine, ethyl-vincamine,

and tabersonine was 1.279, 1.279 mg/L, 0.102 mg/L, respectively (Na *et al*., 2016). In a similar study, 10 endophytic fungal strains were isolated from the roots, stems and leaves of the plant *V. minor*. One fungal strain isolated from the stems was found to produce vincamine although with a relatively lower yield as compared to that of another fungal strain isolated from *N. indicum* (Yin and Sun, 2011).

***Rheum palmatum* L. (Polygonaceae)**

*Rheum palmatum* is a medicinal plant and its air-dried roots have been used in the traditional medicine. *R. palmatum* presents cathartic effect on the digestive movement of the colon, protects the damaged liver, and has antibacterial, anti-inflammation, and anti-aging properties. The most effective biologically active compounds in the roots of the genus *Rheum* are anthraquinones including emodin, rhein, physcion, aloe-emodin. Pharmacological tests revealed that rhein can alleviate pain and fever and inhibits inflammation (You *et al*., 2013).

Fourteen endophytic fungal strains have been isolated from *R. palmatum*: 12 strains were isolated from the root, 2 strains from the stem. The strain R13, isolated from the roots, was capable to produce the bioactive compounds rhein and emodin. The yield of rhein in R13 can reach 5.67 mg/L (You *et al*., 2013).

***Forsythia suspensa* (Thunb.) Vahl. (Oleaceae)**

The main chemical constituents of *F. suspensa* are composed of lignans including phillyrin and forsythiaside, triterpenic acids including oleanolic acid and ursolic acid. Phillyrin was reported to have various biological activities such as antioxidant, anti-inflammatory, anti-hyperlipidemia and antipyretic activities (Qu *et al*., 2008). Studies on phillyrin have shown its presence mainly in the leaves and fruits of the plant *F. suspensa* (Piao *et al*., 2008).

A total of 24 fungal strains were isolated from stems, leaves and fruits of *F. suspensa* and screened for phylirin production. One strain *Colletotrichum gloeosporioides* isolated from the fruits was found to produce the active constituent phillyrin as was judged by TLC, HPLC and HPLC-MS analysis (Zhang *et al*., 2012).

***Miquelia dentata* Bedd. (Icacinaceae), *Camptotheca acuminata* Decne. (Nyssaceae) and *Nothapodytes nimmoniana* (Graham) Mabb. (Icacinaceae)**

Camptothecine (CPT), a quinoline indole alkaloid and its analog, 10-hydroxy camptothecine (10-OH-CPT) are potent inhibitors of the eukaryotic topoisomerase I and are currently used as efficient anticancer drugs against a broad band of tumor types such as small lung and refractory ovarian cancers. (Kai *et al*., 2015). CPT and 10-OH-CPT are naturally produced by several plant species of the Asterid clade. Among them however, the major sources of commercial CPT in the world market are *Camptotheca acuminata* and *Nothapodytes nimmoniana* (Uma Shaanker *et al*., 2008). Exceptional high levels of CPT and 10-OH-CPT are also found in the fruits and seeds of *Miquelia dentata* (Ramesha *et al*., 2013).

Twenty-three fungal isolates were obtained from different fruit parts of *M. dentata*. All fungal isolates produced CPT though in varying quantities (Shweta *et al*., 2013). Three fungal species, *A. alternata*, *Phomopsis* sp. and *Fomitopsis* sp., were identified as CPT-producers with the highest yield of CPT being obtained from *A. alternata* (73.9 μg/g DW) (Shweta *et al*., 2013). CPT-producing endophytic fungi have also been isolated from *C. acuminata* (Pu *et al*., 2013) and *N. nimmoniana* (Bhalkar *et al*., 2016).

## Biochemical convergence or horizontal gene transfer confer the ability to the endophytic fungi to produce the same bioactive compounds as their host

The discovery of endophytic fungi producing the same or similar bioactive compounds as their hosts raises the question as to whether parallel pathways evolved sim-

ply because each lineage has benefitted from making a given compound completely independently of the other or whether horizontal gene transfer (HGT) events took place between the fungi and the plant.

There is precedent for the independent development of the same biosynthetic pathway (biochemical convergence) in fungi or plants and other organisms. For instance, although higher plants and endophytic fungi produce structurally identical GAs, profound differences have been found in the GA pathways and enzymes of plants and fungi (Hamayum *et al*., 2016), e.g. 7-methyl-cyercene-1 found in both the fungus *Leptosphaeria maculans* (anamorph *Phoma lingam*) and the marine mollusk *Ercolania funereal* is produced by distinct enzymes (Cutignano *et al*., 2012). Cyanogenic glucosides linamarin and lotaustralin found in both the moth *Zygaena filipendulae* and their food plant *Lotus japonicus* are biosynthesized by distinct enzyme systems(Jensen *et al*., 2010). However, a horizontal gene transfer event between plants and fungi, although rare, should not be excluded (Richards *et al*., 2009).

Several studies have reported the presence of *Taxus* tree key genes (*ts*,*dbat* and *bapt*) which are involved in plant paclitaxel biosynthesis in taxol-producing endophytic fungi. These results stimulated the conjecture that the origin of this pathway in these two physically associated groups could have been facilitated by horizontal gene transfer (Kusari *et al*., 2014). Other studies, however, provided evidence that microbial taxol genes exist independent of the plant genes (Xiong *et al*., 2013). Recent data support the latter proposal; genome sequencing and analysis of the taxol-producing endophytic fungus *Penicillium aurantiogriseum* NRRL 62431 revealed that out of 13 known plant Taxol biosynthetic genes, only 7 showed low homology(>30%) with genes identified in *P. aurantiogriseum* (Yang *et al*., 2014). Furthermore, polyclonal antibodies against Yaxus TS strongly cross-reacted with a protein of the taxol-producing fungus *Paraconiothyrium* SSM001 grown in liquid culture, whereas PCR analysis did not reveal the presence of *Taxus ts* gene sequences in SSM001 (Soliman *et al*., 2013). Hence, the divergence of the two biosynthetic pathways is supported with conservation only in specific enzyme sites to be important for the activity rather than the whole protein structure. Similar findings have been reported in the case of huperzine A producing endophytes. Their fungal amine oxidase genes have been found to present low similarities to the corresponding plant genes, and only conserved consensus sequences were present by the fungal and plant functional amine oxidase proteins (Yang *et al*., 2014; Yang *et al*., 2016; Zhang et al., *2015*), which supports the co-evolution theory rather than the HGT theory. This has been well established in the case of gibberellin biosynthetic pathways in fungi and higher plants where differences in genes and enzymes indicated converged evolution of GA metabolic pathways (Bömke and Tudzynski, 2009).

The list of taxol producing endophytic fungi is large and encompasses numerous fungi belonging to diverse genera (Stierle and Stierle, 2015). A similar situation appears to hold for CPT-producing fungi (Pu *et al*., 2013) and HupA-producing endophytic fungi (Su *et al*., 2017) suggesting a horizontal transfer of large secondary metabolism gene clusters between fungi. Several studies offer support to this idea; the complete sterigmatocystin gene cluster in *Podospora anserine* was horizontally transferred from *Aspergillus* (Slot and Rokas, 2012). Furthermore, it has been shown that CTP is also produced by a diverse group of endophytic bacteria (Shweta *et al*., 2013; Pu *et al*., 2015) suggesting that bacterial CPT biosynthesis may represent an independently assembled pathway from that in fungi or plants. This may be surprising since converged evolution of the diterpene GA metabolic pathway in plants, fungi and bacteria is well established (Tudzynski *et al*., 2016). Therefore, extensive genome sequencing of the various endophytic fungi will provide an opportunity for a comprehensive study on the phylogenetic origin of fungal and bacterial metabolic pathways.

## Exploring endophytes for sustainable and enhanced production of secondary metabolites

The discovery of endophytic fungi capable of producing the same bioactive compounds as their host medicinal plant has raised the expectation that these compounds could be produced in large scale through fermentation processes, thus meeting the growing demand of the market, while relieving the dependence on their respective endangered host plants for the metabolites. However, this expectation remains hampered primarily by the low yields as well as the attenuation of metabolites production after sub-culturing of fungi (Kusari *et al*., 2011; Kumara *et al*., 2014; El-Elimat *et al*., 2014). The reasons for the attenuation could be attributed to factors that stem from loss of presumed signals provided by the host or co-existing endophytes, resulting in the silencing of genes in axenic monocultures (Sachin *et al*., 2013).

Passage of attenuated CPT-producing endophytic fungi from the host plants restored CPT production in the re-isolated endophytic fungi (Vasanthakumari *et al*., 2015) suggesting that a certain critical signaling may be necessary for the fungus to maintain its endogenous production. Co-cultivation studies of taxol producing fungus *Paraconiothyrium* SSM001 with endophytic fungi isolated from *Taxus* tree revealed an eightfold increase in fungal Taxol production from SSM001 (Soliman and Raizada, 2013). Co-cultivation of the endophytic fungus *Fusarium tricinctum* with the bacterium *Bacillus subtilis*, led to an up to 78-fold enhancement in the accumulation of the constitutively present fungal metabolites (Ola *et al*., 2013). Co-cultivation (mixed fermentation) under optimized conditions of the two CPT-producing fungal species *Colletotrichum fruticola* and *Corynespora cassiicola* isolated from the same host tree *N. nimmoniana* enhanced the yield of produced CPT (Bhalkar *et al*., 2016).

Epigenetic modifications using chemical inhibitors have also been found to be effective in stimulating the transcription of attenuated biosynthetic gene clusters of endophytic fungi (Vasanthakumari *et al*., 2015; Magotra *et al*., 2017), thereby resulting in the enhancement of the production of desired secondary metabolites. Bioprocess engineering strategies such as manipulation of media and culture conditions, co-culture condition, epigenetic modulation, elicitor and or chemical induction, mixed fermentation, and fermentation technology, have been proven promising in alleviating to some extent these obstacles (Venugopalan and Srivastava, 2015).

Upon availability of the endophytic fungal genomes, the putative genes encoding the enzymes involved in the biosynthesis of bioactive compounds could be identified and their function could be verified through transcriptomic, proteomic and metabolomic, RNA interference, gene knockout, and gene over expression. Genome editing technologies implemented for metabolic engineering of filamentous fungi may be applied for triggering the biosynthesis of metabolites. Alternatively, the identified biosynthetic pathway of the corresponding bioactive compounds can be assembled, engineered and then introduced in other genetically tractable microorganisms to increase their yields (El-Sayed *et al*., 2017; Wakai *et al*., 2017).

## Medicinal plant endophytes in Greece

Greece is endowed with a rich biodiversity of medicinal plant species with a long tradition in herbal medicines, and their complex endomicrobiome may be directly and indirectly responsible for the production of a wealth of explored and unexplored bioactive compounds. Thus, it is expected that many new or known products for medicine may emerge through the exploration of the endophytes of these medicinal plants. We are currently isolating fungal and bacterial endophytes from indigenous medicinal plant species in the genera such as *Fritillaria*, *Hypericum*, *Teucrium*, *Calendula*, *Salvia*

as well as *Olea europaea* and the exotic *Nigella sativa* aiming to identify such bioactive compounds.

## Conclusions

Medicinal plants offer an extensive bioresource of new bioactive compounds that have significant potential as antiparasitics, antibiotics, antioxidants, and anticancer agents. During the last 10 years it became apparent that endophytes are capable to produce the same bioactive secondary metabolites as their hosts and therefore there is a tremendous interest of the scientific community towards isolation, characterization and exploitation of endophytic fungi from medicinal plants as was judged by the amount of publications and number of patents (Gokhale *et al*., 2017).

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ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

# Ενδοφυτικοί μύκητες που διαβιούν εντός των φαρμακευτικών φυτών έχουν την ιδιότητα να παράγουν τους ίδιους ή παρόμοιους δευτερογενείς μεταβολίτες με τους ξενιστές τους

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**Περίληψη** Τα φαρμακευτικά φυτά χρησιμοποιούνται εδώ και χιλιάδες χρόνια στην παραδοσιακή φαρμακολογία και ιατρική. Στις μέρες μας, τα φυτά αυτά αξιοποιούνται για την απομόνωση ιδιαίτερα αποτελεσματικών φυτικών φαρμακευτικών ουσιών, με καθόλου ή ελάχιστες παρενέργειες στο χρήστη. Οι φυσικές πηγές φαρμακευτικών φυτών εξαντλούνται σταδιακά με αποτέλεσμα πέραν της οικολογικής διατάραξης από την εξαφάνιση του φυτικού είδους, να κινδυνεύει δραματικά η απόκτηση του βιοδραστικού προϊόντος, το οποίο ούτως ή άλλως βρίσκεται σε χαμηλή συγκέντρωση στο φυτό. Επί παραδείγματι, η ποσότητα των αλκαλοειδών που προέρχονται από φυτά βίνκας και τα οποία χρησιμοποιούνται ως ισχυρά αντικαρκινικά φάρμακα, ανέρχεται στα 3 κιλά ανά έτος δηλαδή απαιτούνται $1.5x10^6$ κιλά ξηρού βάρους φύλλων. Από αυτήν την άποψη, η παρούσα βιβλιογραφική ανασκόπηση αποσκοπεί στο να τονίσει τη σημασία των ενδοφυτικών μυκήτων που διαβιούν εντός των φαρμακευτικών φυτών και οι οποίοι είναι ικανοί να βιοσυνθέτουν τους ίδιους ή παρόμοιους δευτερογενείς μεταβολίτες με τους ξενιστές τους. Επιπλέον, συζητείται η εξελικτική προέλευση των γονιδίων που εμπλέκονται σε αυτές τις μεταβολικές οδούς καθώς και οι προσεγγίσεις που αποσκοπούν στην ενίσχυση της παραγωγής αυτών των μεταβολιτών από ενδοφυτικούς μύκητες.

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