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REVIEW ARTICLE

Biological potential of bioactive metabolites derived from fungal endophytes associated with medicinal plants

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Abstract

Endophytes are endosymbiotic fungi or bacteria that invade and colonize the plant tissue without harming their respective host. Since fungal endophytes live inside the host tissues, their effective isolation and identification is an important step. Endophytic fungi produce bioactive metabolites with unique chemical structures, which may help in improving the physiological condition of both the plants and endophytes. Endophytic fungi are a potent source of all major classes of secondary metabolites including terpenoids, non-ribosomal peptides, polyketides, and alkaloids. With the increase in the number of diseases and human health problems, an intensive search for new sources and effective metabolites is now in progress. The bioactive metabolites produced by the fungal endophytes are competent and effective against various diseases such as cancer, diabetes, infectious diseases, immunological disorders, and cardiovascular diseases. The present review summarizes the efficient approaches used for the screening of fungal endophytes, extraction, and purification of bioactive metabolites along with OMICS based study of endophytes. Along with this, the pharmaceutical importance of novel bioactive metabolites and their efficient production from fungal endophytes using various approaches like genetic engineering, use of elicitors, and precursor feeding are also discussed.

Keywords Fungal endophyte · Genomics · Secondary metabolite · Transcriptomics

Introduction

The life of any organism is inextricably linked to the available macro- or micro-biodiversity and their interaction with each other. Plants have gained importance since ancient times for their significant use in human welfare. For more than 30 years, the metabolites produced from plants have been used by the pharmaceutical industry for drug discovery (Newman and Cragg, 2020). When coming to the protection of medicinal plants, there are many limitations like natural attacks of the pathogen to plants, impedance to grow in an extensive field system, effect of environmental factors and over-exploitation by humans (Verpoorte et al. 2002; Li et al. 2020). Therefore, an alternative is required to produce beneficial metabolites that implicate in improving the physiological state of plants. Endophytes are endosymbiotic microorganisms which are present in various niches and can be grown easily in laboratory conditions, in large amounts, which makes them the best alternative for metabolite production (Ancheeva et al. 2020).

Endophytes can be fungi or bacteria that invade and colonize within the plant tissue without showing any symptoms, either for part or their full life cycle, and show mutualistic interaction with the plants (Wilson 1995). Fungal endophytes are considered to be beneficial for host plants, but some fungi are neutral to them i.e., neither harmful nor beneficial to the host (Backman and Sikora 2008) or some exist in a latent state to become active under certain conditions (Granados et al.

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2020). The global fungal species richness is estimated to be around 2.2 to 3.8 million (Hawksworth and Lücking 2017). It has been evaluated that at least one endophyte is present in each plant (Smith et al. 2008). Fungal endophytes are known to colonize in the tissue of the leaves (phylloplane), flowers (anthosphere), seeds (spermosphere), fruits (carposphere), stems (caulosphere), and roots (rhizosphere) of the host plant (Brader et al. 2017; Shahzad et al. 2016; Saikkonen et al. 2004). Among various proposed theories for host-microbe interaction, "balanced antagonism" illustrates that the fungi overcome chemical and physical barriers to establish the plant-endophyte relationship. It states that asymptomatic colonization of endophyte is a balance of hostility between plants and endophytes and if plant prevents the fungal virulence factors, then only it will show the symptoms (Schulz and Boyle 2006). This relationship not only affects the quantity or quality of bioactive compounds which originate from the medicinal plant hosting the microbes but also helps plants in growth and survival under unfavorable conditions (Chen et al. 2016). Fungal endophytes have complex relationships with their host plant and produce bioactive metabolites. This inter-relationship is closely linked to the distribution and survival of fungi that are directly dependent upon plant fitness. Fungi can produce the same or different compounds when cultured by altering different parameters like aeration, media constitution, temperature, dimension of culture vessel, or by adding different inhibitors (Bode et al. 2002). By varying different cultivation parameters and producing diverse bioactive metabolites by one fungus is an effective strategy, which is termed as OSMAC (one strain-many compounds) method. The OSMAC method is an important tool that helps in the large-scale production of same or different metabolites by activating different silent biogenetic gene clusters of the fungi (Pan et al. 2019). The present review is focused on the recent progress in the area of fungal endophytes and associated bioactive metabolites. In upcoming sections, we also discuss the possible approaches used for the efficient screening, isolation of fungal endophytes, the methods used for the extraction and characterization of their bioactive metabolites. Different classes of fungal endophyte derived bioactive metabolites and their biological activities are also discussed. The present review also discusses about various strategies to enhance the production of the bioactive metabolites, OMICS based approaches to study fungal endophytes and future perspectives.

Isolation of fungal endophytes

Fungal endophytes hold immense potential to yield bioactive metabolites, thus the precise selection of candidate plants for the screening of valuable endophytes is an imperative step. To accomplish the beneficial facets of these endophytes, an adequate isolation process is a prerequisite. The habitats (deserts, rainforests, swamps, and marshes) and ethnobotanical history of endophytes also have significant consideration for selection of host plants. The host plant that is colonized by endophytic fungi ranges from cryptogams (such as algae, bryophytes, and pteridophytes) to phanerogams (angiosperms and gymnosperms) (Paranagama et al. 2007; Zheng et al. 2018; Zhou et al. 2015). Different plant tissues including bark, leaves, fruit, roots, scales, flowers, and stems are harvested from the candidate plants. The plant tissues are collected in an autoclave bag after sampling and washed in running tap water followed by distilled water. Plant tissues are further cut into small pieces (approximately 2–4 mm) and processed for surface disinfection to kill surface microorganisms. Thereafter, the tissues are placed on selection medium like potato dextrose agar (PDA), Czapek's agar (CZA), modified Melin-Norkrans (MMN) medium, corn meal agar (CMA), malt extract agar (MEA), Sabouraud dextrose agar (SDA), water agar (WA), or V-8 agar supplemented with suitable antibiotics (such as penicillin/streptomycin/chloramphenicol/oxytetracycline/novobiocin). Further, the samples are carefully incubated at optimized parameters such as temperature, light, duration, and pH (VanderMolen et al. 2013; Potshangbam et al. 2017).

Further, the fungal isolates are subcultured from spores or hyphal tips to get purified culture. Various fungal isolates fail to sporulate during cultivation, which are referred as "sterile mycelia." Identification of fungal endophytes at the genus or species level is difficult (Wang et al. 2005). The morphological identification of fungal endophytes except "sterile mycelia" attributes to several factors such as shape and size of spore or hyphae, color and texture of colony, conidial attachment, and fungal growth rate.

Molecular identification is carried out using DNA barcodes, in silico tools, and several online databases. DNA barcoding includes the isolation of genomic DNA (gDNA) and its PCR amplification using primers of DNA barcodes (Diaz et al. 2012). Internal transcribed spacer (ITS) is often used as a DNA barcode to identify the fungal isolates. Subsequently, other DNA barcoding markers such as DNA-directed RNA polymerase II (*RPB1* and *RPB2*) subunits, β-tubulin-II (*TUB2*), phosphoglycerate kinase (*PGK*), and translation elongation factor 1α (*TEF1α*) can also be used to identify fungal isolates at species or genus level. Beside these, DNA marker cytochrome-C oxidase subunit I (COX1) is also used as a DNA marker, but it is not as important as other genes like *TUB2* and *TEF1α*. However, ITS based screening is not enough to identify certain fungal strains at species level; thus, it is recommended to use secondary DNA barcodes for precise identification of fungal isolates (Lücking et al. 2020).

Advanced microscopy techniques such as laser microdissection and pressure catapulting (LMPC) techniques can also be used for the isolation of fungal endophytes directly from the plant tissues (Gautam et al. 2016; Kerk et al. 2003; Gautam

and Sarkar 2015; Verma et al. 2019). In LMPC, a microscope is used to visualize the morphology of hyphae or desired tissue and a laser beam is used to cut. Further, the DNA could be extracted from the hyphae for the molecular identification of the individual fungal endophytes. The stepwise procedure for isolation of bioactive metabolites from fungal endophytes and their biological application is presented in Fig. 1.

Extraction of secondary metabolites from fungal endophytes

Fungal endophyte derived metabolites could be extracted by liquid fermentation. Fermentation of fungal culture could be carried out in various liquid media, for example, Potato Dextrose Broth (PDB), Malt Extract Broth (MEB), Czapek Dox Broth (CDB), Peptone Yeast Glucose (PYG) Broth, and Yeast powder soluble starch (YpSs). The culture flask containing selected liquid media and fungal isolate is placed in an incubator at 25 ± 2 °C with constant shaking for 15–21 days (Sharma et al. 2016). Solvent partitioning is done by filtering the broth and extracting the compound with a solvent like ethyl acetate, dichloromethane, hexane, chloroform, n-butanol, methanol, or as per the need of the experiment. The organic phase is collected, and evaporation of the solvent is done using rotatory evaporator under reduced pressure at 35–40 °C to obtain the crude extracts (Gao et al. 2017; Nevalainen et al. 2014). Later, the fungal extracts are dissolved in a solvent like dimethyl sulphoxide (DMSO) or methanol for the screening of their biological activities (Sharma et al. 2016). The biological activities of extracted bioactive compounds are performed either by the use of different immunoassays such as 2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH) for antioxidant activity, or through different in vitro biological assays such as (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) (MTT) for anticancer activity, serial dilution assay for antimicrobial activity, and chloroquine bioassay for antimalarial activity (Hamzah et al. 2018; Yadav et al. 2014; Khan et al. 2019; Calcul et al. 2013). The purification and characterization of extracted bioactive compounds can be performed after checking their bioactivities.

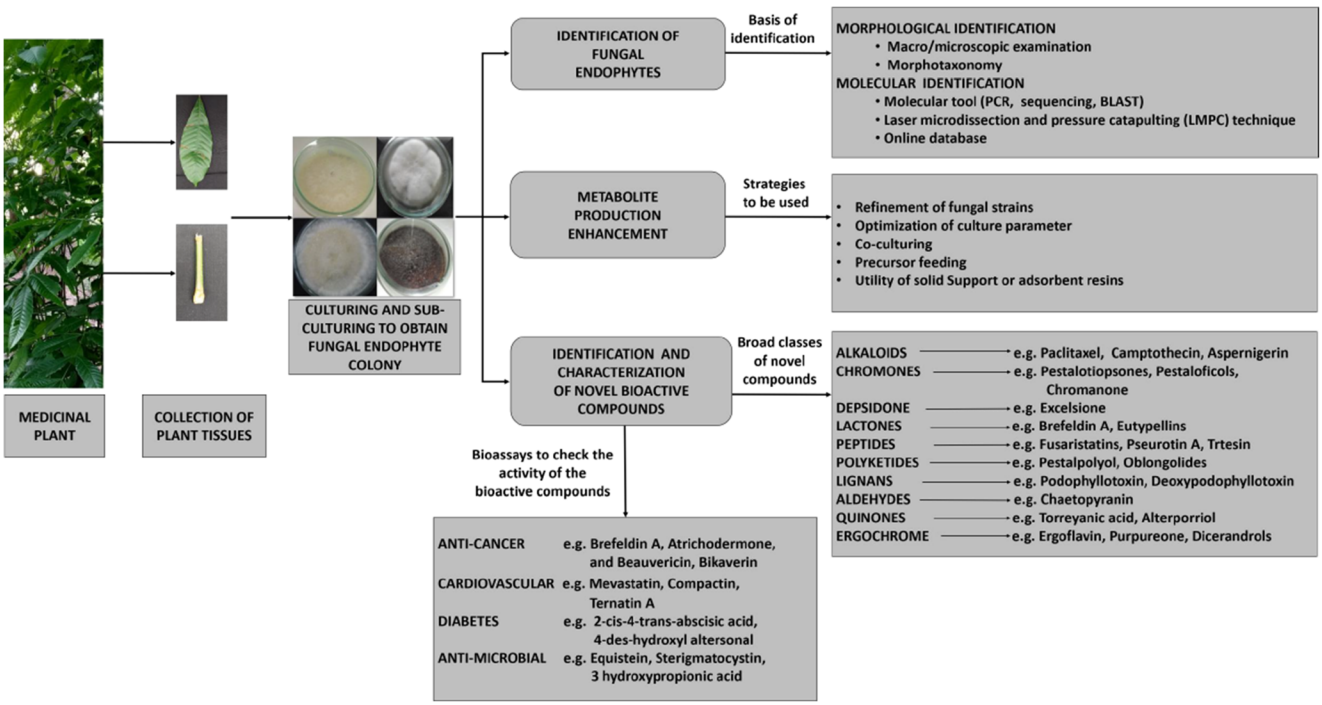

Fig. 1 An overview of the isolation and characterization of fungal endophytes from medicinal plants along with the implication of its bioactive metabolites in human health care. Fungal endophytes are isolated from different parts of the medicinal plant like leaves, stem, fruits, and roots. Plant tissues are cultured in growth media after sterilization and then subcultured to obtain specific fungal endophytes. For the identification of the endophytes, morphological methods like macro and microscopic description of different parts of fungi are studied, while molecular techniques include isolation of genomic DNA and PCR amplification of gDNA using primers of DNA barcodes followed by BLAST. Several strategies (refinement of fungal strains, optimization of culture parameter, co-culturing, precursor feeding, and utility of solid support or adsorbent resins) are applied for the improvement of fungal endophytes which leads to enhanced production of bioactive metabolites. For the extraction, purification, and characterization of different bioactive metabolites, various instrumentation techniques like HPLC, HPTLC, NMR, IR, and GC-MS are used. The isolated bioactive metabolites can be of any broad classes like alkaloids, quinones, chromones, depsidone, lactones, lignans, polyketides, aldehyde, or ergochromes. Bioactive metabolites are further evaluated for various health ailments such as cancer, cardiovascular, diabetes, and other diseases

Separation and purification techniques

Since the fungal crude extracts contain various bioactive metabolites with different polarities and molecular masses, it is important to separate and purify the bioactive metabolites. Diverse chromatography techniques can be used for this purpose. Adsorption chromatography uses a stationary phase (silica gel, sephadex LH20) and a mobile phase (methanol, ethanol, hexane, ethyl acetate) for isolation and purification of metabolites as a different fraction (Verma et al. 2014; Bogner et al. 2017). Gel permeation chromatography uses Sepharose-6B, Sephacryl, and Sephadex-G100 as gel filtration column and is also used to purify the metabolites (Liu et al. 2017). For qualitative and quantitative analysis, preparative reverse-phase ultra-performance liquid chromatography (RP-UPLC) or preparative reverse-phase high-performance liquid chromatography (RP-HPLC) can also be used (Reis et al. 2018). The pure solvent or a mixture of different solvents can be used as mobile phase depending on the need of the experiment.

Structural elucidation of the purified metabolite

The structure of the purified bioactive metabolite can be determined by using spectroscopic and spectrometric techniques. UV-VIS spectroscopy helps to know the electronic features of purified bioactive metabolite like chromophore characteristics (Qin et al. 2009). A reliable and sensitive liquid chromatography-mass spectrometry (LC-MS) method is used for the identification of the known and unknown group of bioactive metabolites. By finding the large numbers of parent ions present in the extracts, LC-MS identifies their chemical features (Arora et al. 2016). Infrared spectroscopy is used to determine the functional group of the compound. The characterization is done by UV-visible spectroscopy, infrared (IR) spectroscopy, Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) spectroscopy, and by coupling methods such as LC-MS or electrospray ionization-mass spectrometry (ESI-MS). NMR spectroscopy identifies hydrogen and carbon in the compound and gives structural elucidation of the bioactive compounds (Gao et al. 2017; Balagurunathan et al. 2020). Various hyphenated techniques like high-resolution electrospray ionization mass spectrometry (HR-ESI-MS), electrospray ionization mass spectrometry (ESI-MS), and gas chromatography-mass spectrometry (GC-MS) can be also used for quantitative analysis of fungal endophyte derived bioactive metabolites (Sharma et al. 2016; Tan et al. 2019; Mishra et al. 2017).

OMICS based study for fungal endophytes

Most of the study on biological activities and host-endophyte relationship are known to be carried out using traditional approaches; however, the molecular and biophysiochemical aspects of host-endophyte relationship remain obscure. To attain deeper insight to research on fungal endophytes, an OMICS based approach appeared to be a promising stratagem. Generally, bioactive metabolites are synthesized via multienzyme pathways, and the proteins including single pathway are often encoded by biosynthetic gene cluster (BGC). Therefore, multidisciplinary approaches such as molecular biology, biochemical assays, bioinformatics, genomics, proteomics and metabolomics are required for a better understanding of the true potential of BGCs of fungal endophytes. The OMICS based approach offers enormous data that enhance the understanding for diverse aspects including evolution, phylogenetic lineage, fungal diversity, functional gene expression, protein turnover and post-translational modification. Some of the major OMICS based approaches which can be used in addressing fungal endophyte research are discussed below.

Genomic studies

With the advent of NGS, the genome sequencing has immensely progressed and uncovered new dimensions of genomic or comparative genomics studies. The continuous increase in fungal genome sequencing data unveils the identification of bioactive metabolite producing gene or its associated network. The bioactive metabolite producing gene clusters can be identified using bioinformatic algorithms such as FungiFun (Priebe et al. 2011), antiSMASH (Medema et al. 2011) and SM Unknown Regions Finder (SMURF) (Khaldi et al. 2010). For instance, the gene encoding polyketide synthases (PKS) and its adjacent genes were predicted using the aforementioned tools, which leads to the identification of gene clusters associated with bioactive metabolite production in the fungal genome. An endophytic strain of *Pestalotiopsis fici*, associated with *Camellia sinensis*, is known to produce structurally unique bioactive metabolites such as pestalofones, pestaloficiols, chloropupukeananin, pestalodiols, and chloropestolides. The bioactive metabolites isolated from *P. fici* have antifungal and tumor cytotoxic activity. Genomic studies performed on *P. fici* revealed the presence of carbohydrate-active enzymes (CAZymes) and gene clusters, responsible for secondary metabolite synthesis (Wang et al. 2015). The draft genome sequence analysis of an endophytic *Gaeumannomyces* sp. strain JS-464, associated with *Phragmites communis*, revealed the production of several bioactive metabolites with significant nitric oxide reduction activity (Kim et al. 2017). Transcriptome and genome assembly of *Lolium perenne* has provided evidence for horizontal gene

transfer of an enzyme β-1,6-glucanase of fungal origin, responsible for the degradation of the fungal cell wall (Shinozuka et al. 2017). In a recent study, comparative genome analysis of *Sarocladium brachiariae* and *S. oryzae* revealed that *S. brachiariae* has 33.3% more fungal cell wall degradation related carbohydrate-active enzymes than *S. oryzae*. Synteny analysis performed between *S. oryzae* and *S. brachiariae* for non-ribosomal peptide synthetases (NRPS), polyketide synthases (PKS), and hybrid (PKS-NRPS) gene clusters showed that 63.5% of tested clusters show poor synteny, while only 37.5% show good synteny. Their study suggests that *S. brachiariae* could be a potential source for the production of a variety of bioactive metabolites (Yang et al. 2019b).

In addition to the whole genome studies, various metagenomic studies on fungal endophytes are also reported. Genomics and metagenomics studies are used to understand the presence or absence of specific genes in the individual species or in a community, respectively. Metagenomics also helps to elucidate the beneficial characteristics and metabolic potential of fungal endophytes. A previous report has identified the complete mitochondrial (mt) genome of the endophyte *Epichloë festucae* var. *lolii* (GenBank accession number -KF906135), which is known to play a significant role in plant stress (Ekanayake et al. 2013). In another study, using Illumina based sequencing approach, fungi associated with the root tissues of a monodominant forest depicts a network representing patterns of co-occurrence of symbiotic associations (Toju et al. 2016).

Transcriptomic studies

Some new information on gene expression modulation related to plant-fungal mutualism can be achieved through transcriptomic studies. Studies on the transcriptome can be performed in two ways either by using the existing whole genome sequences or by de novo assembly. Both approaches can produce significant information on the gene expression status. The upregulation of genes responsible for pyrimidine metabolism was revealed by transcriptome analysis of the fungus *Epulorhiza* sp., associated with *Anoectochilus roxburghii* (Li et al. 2012a). The differential expression of antifungal protein producing genes was shown in the endophyte *Epichloë festucae*, associated with *Festuca rubra* (Wang et al. 2019a). In one of the studies, a combined approach of transcriptomics, genomics, and metabolomics of *Ascocoryne sarcoides* was performed for the identification of genes responsible for biodegradation of cellulose (Gianoulis et al. 2012). Recently, transcriptomic analysis of a human liver cancer cell line (HepG2) treated with partially purified fractions of the fungal extract revealed the differential expression of various metabolism and cancer pathway genes. The genes associated with key biological processes such as necroptosis, focal adhesion, HIF-1 signaling, transcriptional misregulation of cancer, DNA replication, p53 signaling, cell cycle and drug metabolism exhibited differential expression (Blessie et al. 2020). These transcriptomic details have been used to determine the possible functions of genes to predict the metabolic pathways and traits important for fungal lifestyle. The transcriptome analysis also helps to predict the role and regulation of the genes involved in the reactive oxygen species (ROS) detoxification and biosynthesis of bioactive metabolites associated with fungal endophytes.

Proteomic studies

Fungal endophytes secrete proteins, which are known to play a significant role in plant cells during symbiosis. Proteomics provides a clear understanding of plant-endophyte interaction. A proteome-based study is reported for the fungal endophyte *Serendipita indica* (formerly known as *Piriformospora indica*) which colonizes root tissues of most of the terrestrial plants. A total of 45 differentially expressed proteins involved in ROS scavenging, photosynthesis, signal transduction, metabolisms, and plant defense response has been identified using mass spectrometry. In order to increase the yield of cellular protein extraction from fungal endophyte an efficient protocol has been established which has been used for to perform two-dimensional gel electrophoresis (2D-PAGE) (Yadava et al. 2015).

Recently, in-depth proteomics was conducted to characterize the molecular physiology of *Serendipita indica* and *Brassica napus* host plant relationship, to show its potential role in symbiosis and plant development. The differential proteomics was evaluated using a label-free quantitative technique LC-MS/MS of *Serendipita indica* treated plant and control plants. Out of 8123 assessed proteins, 34 were downregulated and 12 were upregulated. KEGG pathway and GO analysis of differentially expressed proteins revealed that gene clusters responsible for symbiotic signaling, stress responses, and biosynthesis of bioactive metabolites were enhanced (Shrivastava et al. 2018).

Metabolomic studies

Presently, research is primarily focused on fungal endophytes having unique property to produce structurally diverse and therapeutically important bioactive metabolites. Mostly, the proteomic studies are based on the multivariate analysis using GC-MS or LC-MS. Comparative study of metabolome of *Aspergillus terreus*, associated with *Opuntia ficus-indica*, was grown under 11 different culture conditions using rice-based media, solid agar, or broth cultures. LC-MS analysis of

the organic extract revealed extreme differences in the metabolic profiles and identified one new bioactive metabolite 7-desmethylcitreoviridin and 16 known metabolites (Adpressa et al. 2016). In another study, untargeted metabolomic analysis was used to analyze the effect of histone deacetylase (HDAC) inhibition on the production of bioactive metabolite produced by an endophytic *Aspergillus nidulans*. Quantitative and differential analyses revealed that 61 and 47 metabolites were upregulated and downregulated, respectively, by more than 100-fold. Transcriptomic elucidation reveals that the biosynthetic machinery is usually upregulated due to the presence of HDAC inhibitor, while their result suggests that the responses at secondary metabolome level are quite complex than that of a global increase in richness of bioactive metabolites. Lastly, they had shown that *A. nidulans* can produce fellutamides, which inhibit protease activity (Albright et al. 2015). In one of the reports, a systematic metabolomic analysis was performed to evaluate the potential impact of DNA methyltransferase (DNMT) and HDAC inhibition on bioactive metabolite produced by an endophytic *Dothiora* sp. (González-Menéndez et al. 2016). In another study, metabolic tools and dereplication analysis was performed using high-resolution electrospray ionization mass spectrometry (ESI-MS) on liquid and solid culture extracts a *Curvularia* sp. associated with the plant *Terminalia laxiflora* for a comparative study of bioactive metabolite. Multivariate analysis of the mass spectral data of both fungal and host plant extracts was analyzed by principal component analysis (PCA). PCA loading plot identified a bioactive metabolite found on 30-days culture extract showing 95% inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in myelogenous leukemia cell line (K562) (Tawfike et al. 2017). Proteomics and transcriptomics studies unite the linear predictive potential of the genome, while, the aforementioned metabolomic studies signify the non-linear, final bioactive products of the genome, arising from the complex system(s) that regulate the expression of the genome.

Fungal endophytes as a source of bioactive metabolites

Fungal endophytes produce different types of bioactive metabolites that play an essential role in host plant interaction, symbiosis regulation, and defense (Schulz and Boyle 2005). Fungal metabolites are important for humans as they are used in the management of various health-related problems (Rai et al. 2021). For example, enfumafungin, an antifungal drug, isolated from an endophyte of *Juniperus communis*. The compound showed activity against *Aspergillus* and *Candida*, including in vivo activity in a mouse model infected with *Candida septicemia* (Peláez et al. 2000). A semisynthetic derivative of enfumafungin named ibrexafungerp (formerly known as SCY-078) has been pre-approved by Food and Drug Administration (FDA) as an alternative therapeutic option for the treatment of mild to chronic vulvovaginal candidiasis (VVC) (Davis et al. 2020). Ibrexafungerp is a triterpenoid antifungal agent which can be administered both intravenously and orally due to its high bioavailability. Similar to echinocandins, ibrexafungerp causes a decrease in the level of (1,3)-β-D-glucan polymers and weakens the fungal cell wall (Pfaller et al. 2013). Beside similar mode of action, ibrexafungerp shows significant in vitro activity against fungal pathogens of *Candida* sp. with fks1 and fks2 mutations, whereas such mutations cause resistance among *C. glabrata*, *C. auris*, and *Aspergillus* spp. against the drug echinocandin (Larkin et al. 2017; Nunnally et al. 2019; Pfaller et al. 2017). Thus, it can be concluded that ibrexafungerp shows a different target site avidity than echinocandins. After several in vivo and in vitro studies, ibrexafungerp has undergone for clinical studies. A total of nineteen phase 1, three phase 2, and two phase 3 clinical-based studies are completed for ibrexafungerp. First study of phase 2 (clinicaltrials.gov: NCT02679456) was performed as proof-of-concept on women having mild to severe VVC (Roman et al. 2017). While, the second study of phase 2, DOVE (clinicaltrials.gov: NCT03253094) was conducted on the female subjects to determine the ranging-dose of the compound (Cadet et al. 2019). In both the studies of phase 3 (VANISH-303 and VANISH-306) (https://clinicaltrials.gov: NCT03734991 and NCT03987620), the mycological eradication and clinical therapy (a significant improvement in the symptoms of VVC after 10 days) were substantially better with ibrexafungerp in comparison to placebo (Azie et al. 2020). However, the side effects related to gastrointestinal problems appear after administration of ibrexafungerp in more than 1200 patients and a control subject. In the coming future, the clinical importance of ibrexafungerp will be defined, but the development towards the targeted therapeutics against fungal infections caused by resistant fungal species or oral step-down therapy is needed.

Nodulisporic acids (NAs) are unique indole diterpenes that have been under development as antiparasitic and insecticidal drug for use in veterinary medicine (Ondeyka et al. 1997). Nodulisporic A modulates the glutamate-gated ion channel, an invertebrate specific channel and exhibits insecticidal activity against fleas (Smith et al. 2000). These compounds were found to be exclusively produced by a monophyletic lineage of fungal endophytes, which were previously classified in the genus *Nodulisporium*. Bills et al. (2012) could find the sexual state of these endophytes and thereby elucidated the life cycle of the fungus, which is now classified in the genus *Hypoxylon* (Hypoxlaceaee) and was named *H. pulicicidum* (Bills et al. 2012). The genus *Diaporthe* and its asexual state *Phomopsis* (name not used anymore) is an excellent source of wide range of bioactive metabolites (Chepkirui and Stadler 2017). Endophytic fungi can produce plant hormones like

gibberellins and produce similar metabolites as their relatives like saprotrophs and pathogens (Promputtha et al. 2007; MacMillan 2001; Masi et al. 2018). Brief descriptions about the metabolites which are produced from fungal endophytes along with its uses are discussed below.

Alkaloids

Alkaloids are a naturally occurring group of compounds that are therapeutically used as antimicrobial, antitumor, antimalarial, vasodilatory, anti-hyperglycemic, and anti-asthmatic. Here, we have discussed a few examples such as phomoenamide, atrichodermone, asperimides, and ascomylactams.

GKK1032C (Fig. 2a), a new alkaloidal compound, was extracted from the endophytic fungus *Penicillium* sp. CPCC 400817, associated with mangrove plant. GKK1032C exhibits antibacterial activity with MIC value of 1.6 μg/mL against the bacterium *Staphylococcus aureus* (Qi et al. 2019). Penicinoline (Fig. 2b), a pyrrolyl 4-quinoline alkaloid, isolated from an endophytic *Penicillium* sp. showed cytotoxic activity against HepG2 and a 95-D cell line with an IC_{50} value of 6.5 μg/mL and 0.57 μg/ml, respectively (Shao et al. 2010). Penicinoline was also evaluated against the *Plasmodium falciparum* malarial parasite and showed excellent antimalarial activity with an IC_{50} value of 1.56 μM (Naveen et al. 2017). Phomoenamide (Fig. 2c), an amide derivative isolated from *Diaporthe* sp. (originally reported as *Phomopsis* sp. PSU-D_{15}), an endophytic *Garcinia dulcis* Kuiz leaves. The antibacterial activity of phomoenamide was shown against *Mycobacterium tuberculosis* with a MIC value of 6.25 μg/ml (Rukachaisirikul et al. 2008). Three novel compounds, atrichodermone A (Fig. 2d), atrichodermone B (Fig. 2e), and atrichodermone C (Fig. 2f), were isolated from an endophytic *Trichoderma atroviride*, associated with *Lycoris radiata* plant. Atrichodermones A, B, and C showed cytotoxic activity against U967 and HL60 cell lines and anti-inflammatory effect against IL-1β and TNF-α (Zhou et al. 2017). From the plant *Michelia champaca*, *Colletotrichum gloeosporioides* was isolated, and the fermentation product 2-phenyl 1H-indol-3-yl-acetate was extracted. 2-Phenyl 1H-indol-3-yl-acetate displayed antifungal activity against *Cladosporium sphaerospermum* and *Cladosporium cladosporioides* (Chapla et al. 2014). Four aromatic butenolides, asperimides A-D were obtained from the culture of an endophytic *Aspergillus terreus* associated with the leaves of *Suriana maritima* L. Compound asperimide C (Fig. 2g) and D (Fig. 2h) exhibited anti-inflammatory activity in lipopolysaccharide induced RAW264.7 cells with an IC_{50} value of 0.78 and 1.26 μM, respectively (Liao et al. 2018). Three new macrocyclic alkaloids, ascomylactams A–C, were extracted from the *Didymella* sp. fungal endophyte of mangrove plant. The cytotoxic activity of ascomylactam A (Fig. 2i) was seen maximum against NCI-H460 human cancer cell line with IC_{50} value of 4.4 μM (Chen et al. 2019). All these promising results enable the researchers and pharmaceutical industries to explore more about the fungal endophytes as a novel source to produce alkaloids at a lower cost.

Ergochromes

Ergot alkaloids are known worldwide as toxic metabolites produced by the ergot fungi, a genus of *Claviceps*. Ergot sclerotia contain biphenyl pigments known as ergochromes. One of the members of ergochrome is ergoflavin (Fig. 3a) having

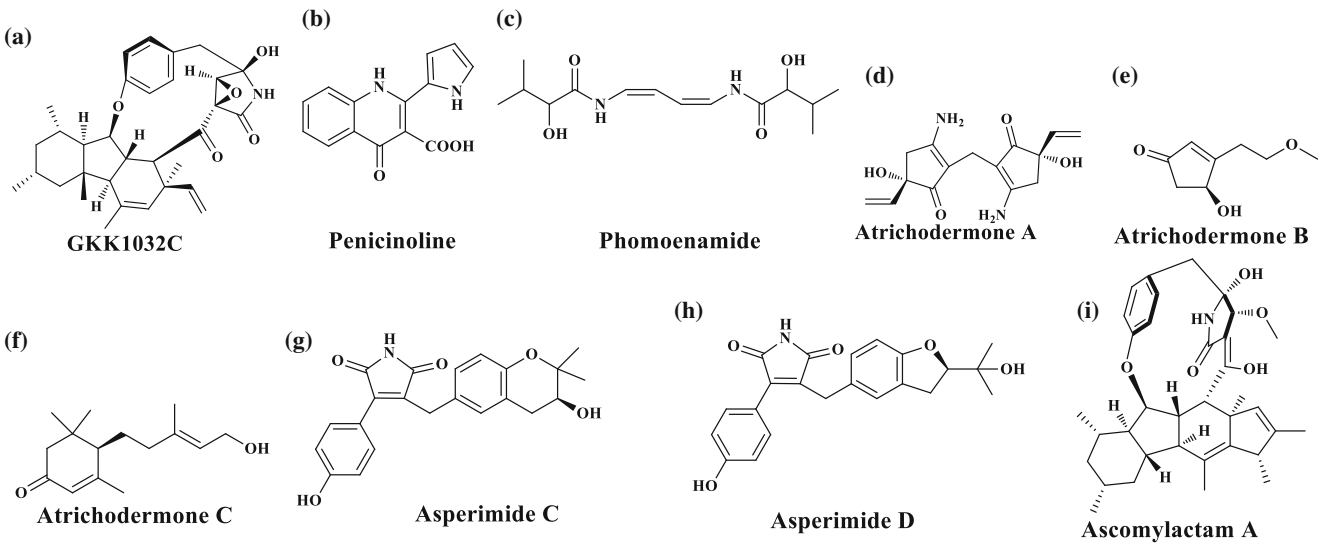

Fig. 2 Chemical structure of alkaloids. **(a).** GKK1032C**; (b).** Penicinoline**; (c).** Phomoenamide**; (d).** Atrichodermone A**; (e).** Atrichodermone B**; (f).** Atrichodermone C**; (g).** Asperimide C**; (h).** Asperimide D**; (i).** Ascomylactam A

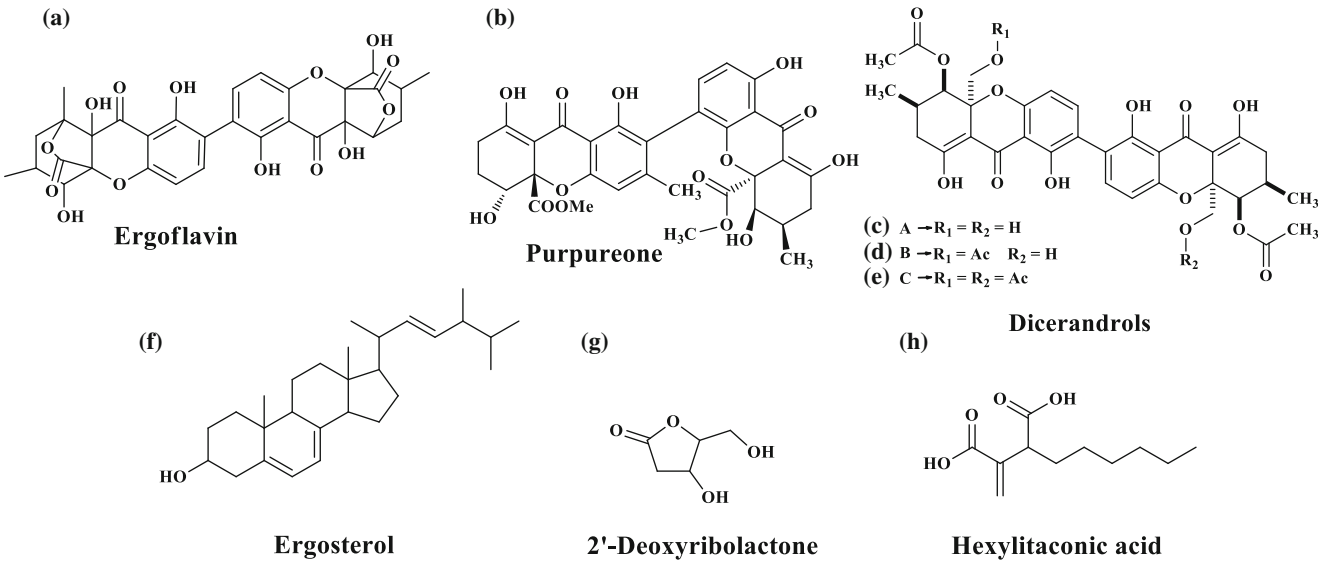

Fig. 3 Chemical structure of ergochromes. **(a).** Ergoflavin**; (b).** Purpureone**; (c).** Dicerandrol A**; (d).** Dicerandrol B**; (e).** Dicerandrol C**; (f).** Ergosterol**; (g).** 2'-Deoxyribolactone**; (h).** Hexylitaconic acid**; Me:** CH_3**; Ac:** Acetyl group

molecular formula $C_{30}H_{26}O_{14}$. The biological role of ergoflavin is to inhibit TNF-α and IL-6 (Hiragun et al. 2005). From the ethanolic extract of *Purpureocillium lilacinum*, associated with *Rauvolfia macrophylla*, an ergochrome derivative purpureone (Fig. 3b) was extracted. Purpureone showed anti-leishmanial activity with an IC_{50} value of 0.63 μg/mL against *Leishmania donovani*, antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Providencia stuartii*, *Paenibacillus* (formerly known as *Bacillus*) *cereus*, and *Listeria monocytogenes* and antiprotozoal action against *Trypanosoma* sp., and *Plasmodium falciparum* (Lenta et al. 2016). From the stem of endangered mint plant *Dicerandra frutescens*, an endophytic fungus *Diaporthe* sp. (originally reported as *Phomopsis longicola*) was isolated, which led to the synthesis of the dicerandrol A (Fig. 3c), dicerandrol B (Fig. 3d) and dicerandrol C (Fig. 3e). Dicerandrol A, B and C exhibited cytotoxic activity against human tumor cell line HCT-116 and A549 (Wagenaar and Clardy 2001). Three compounds ergosterol (Fig. 3f), 2′-deoxyribolactone (Fig. 3g) and hexylitaconic acid (Fig. 3h) were extracted from the culture of *Curvularia* sp. T12 associated with *Rauwolfia macrophylla*. Ergosterol, 2′-deoxyribolactone, and hexylitaconic acid showed inhibitory activity against acetyl cholinesterase with IC_{50} value of 1.52μM, 1.93 μM and 1.54 μM, respectively (Kaaniche et al. 2019).

Peptides

Peptide biosynthesis occurs either ribosomally or non-ribosomally. Ribosomal biosynthesis includes formation of mature peptides which is ribosomally synthesized and by the post-translational modifications of peptide precursor (RiPP). Non-ribosomal biosynthesis is mediated by non-ribosomal peptide synthetases (NRPSs). NRPS consist of three functional domains which is adenylation domain (for recognizing amino acid derivative by adenylation process), peptidyl carrier domain (that helps in binding of 4′-phosphopantetheine and a thioester) and condensation domain (help in peptide bond formation) (Bills and Gloer 2016). RiPP are generally found in Ascomycota group of fungi. Two cyclic lipopeptides fusaristatin A (Fig. 4a) and fusaristatin B (Fig. 4b) were obtained from a *Fusarium* sp. YG-45 isolated from stem of *Maackia chinensis*. Fusaristatins A and B exhibited an inhibitory effect on topoisomerase-I and II and showed an apoptotic effect on LU65, a lung cancer cell line (Shiono et al. 2007). PF1022A (Fig. 4c) is a cyclooctadepsipeptide compound which was isolated from *Rosellinia* species associated with the Japanese tea plant and showed antihelminthic activity both in vitro and in vivo (Helaly et al. 2018).Its semisynthetic analog emodepside is a marketed antiparasitic drug (Scherkenbeck et al. 2002). This is the only metabolite from fungal endophytes which is commercially available as drug. The nematocidal PF1022A was purified first time from ascospore derived fungal isolate *Rosellinia corticium* but its production is seen from different strains of *Rosellinia* and their related genus *Astrocystis* (Wittstein et al. 2020). Astin, a cyclopeptide, exhibits cytotoxic effect as it binds with human regulatory protein and stimulates the interferon genes. Astin A (Fig. 4d) was produced by the endophyte *Cyanodermella asteris* associated with the aster plant (Schafhauser et al. 2019). Trichomide A (Fig. 4e) and trichomide B (Fig. 4f), a cyclodepsipeptide, isolated from *Trichothecium roseum* associated with the plant *Imperata cylindrica*. Trichomides A and B act as a valuable immunosuppressant as it decreases the

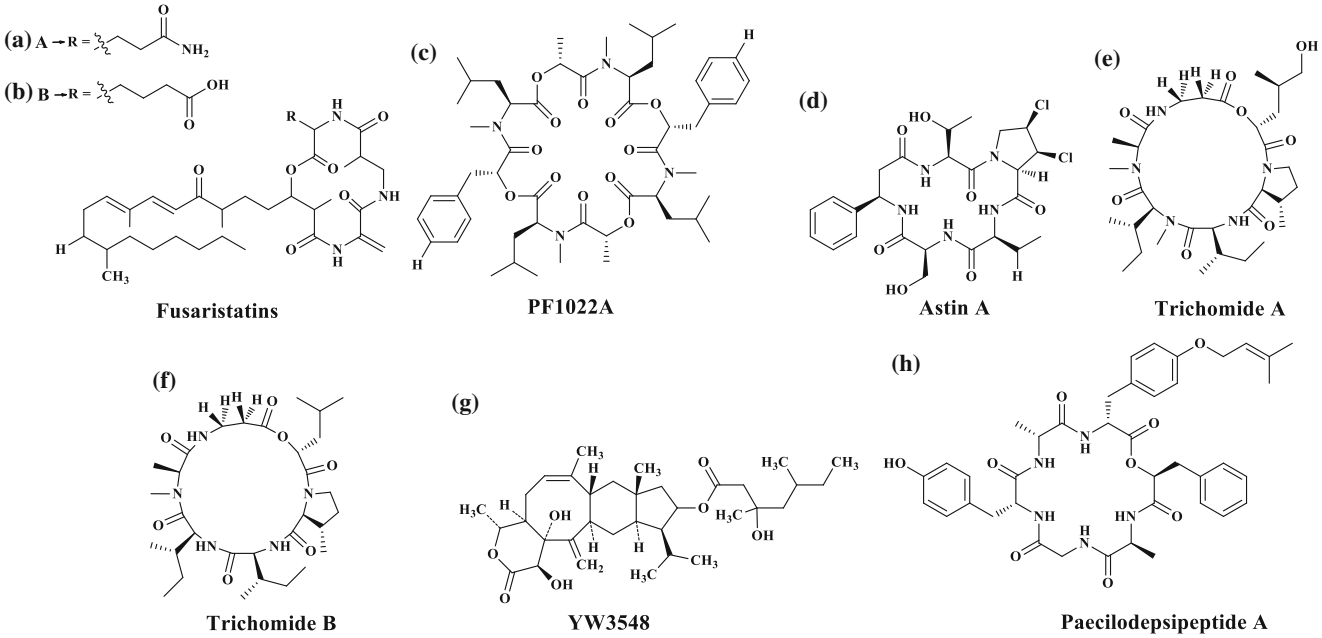

Fig. 4 Chemical structure of peptides. **(a).** Fusaristatin A**; (b).** Fusaristatin B**; (c).** PF1022A**; (d).** Astin A**; (e).** Trichomide A**; (f).** Trichomide B**; (g).** YW3548**; (h).** Paecilodepsipeptide A

expression of anti-apoptotic protein, Bcl-2 (B-cell lymphoma 2) with mild effects on the levels of CD25, CD69, and p-Akt (Zhang et al. 2013). A sester-terpenoid YW3548 (Fig. 4g) and a cyclic peptide paecilodepsipeptide A (Fig. 4h) was extracted from the culture broth of fungal endophyte LHL10 associated with *Cucumis sativus* (cucumber plant). YW3548 exhibited enzyme inhibitory activity against α-glucosidase and urease with IC_{50} value of 61.80 μg/g and 75.68 μg/g, respectively. Paecilodepsipeptide A also showed enzyme inhibitory activity against α-glucosidase and urease with IC_{50} value of 74.25 μg/g and 190.5 μg/g, respectively (Bilal et al. 2018).

Polyketides

Polyketides are a group of bioactive metabolites which are synthesized by a large group of multifunctional enzymes which is polyketide synthases (PKSs) (Bills and Gloer 2016). Some of the polyketide like gibberellins are obtained from both plants and fungi. A polyketide pestalpolyol (Fig. 5a) extracted from the endophyte *Pestalotiopsis clavispora* associated with the mangrove plant *Rhizophora harrisonii* exhibited cytotoxic activity against mouse lymphoma cell line (L5178Y) (Pérez Hemphill et al. 2016). Another polyketide compound derivative, oblongolide (Fig. 5b) was isolated from endophytic *Diaporthe* sp. (originally reported as *Phomopsis* species). Oblongolide exhibited anti-HSV activity and cytotoxic activity against the non-malignant cell lines, BC, KB, and NCI-H187 (Bunyapaiboonsri et al. 2010). A polyketide compound isolated through fermentation from *Cladosporium* sp. associated with *Excoecaria agallocha* mangrove plant showed scavenging activity (Wang et al., 2018). Four polyketides namely bellidisins A-D were isolated from the culture of the endophyte *Phoma bellidis* associated with *Tricyrtis maculata* plant. Out of them, bellidisin D (Fig. 5c) showed moderate but significant cytotoxicity against various cell lines (Wang et al. 2019b). Six polyketides named aplojaveediins A-F were isolated from *Aplosporella javeedii* associated with *Orychophragmus violaceus*. Aplojaveediin A (Fig. 5d) showed significant antifungal activity against *Candida*

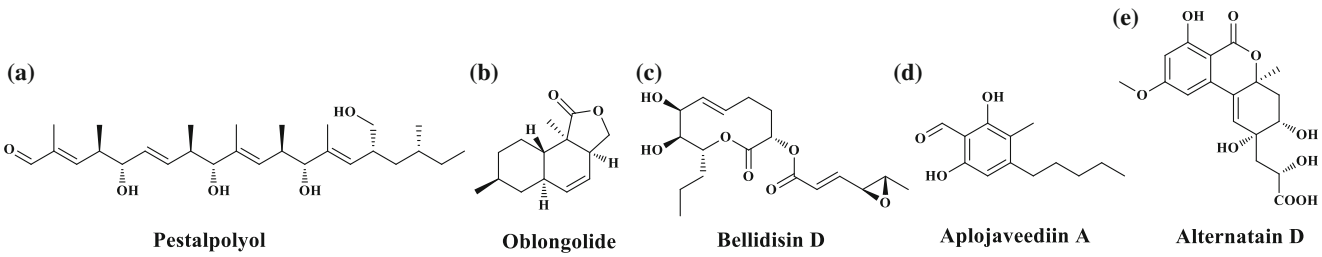

Fig. 5 Chemical structure of polyketides. **(a).** Pestalpolyol**; (b).** Oblongolide**; (c).** Bellidisin D**; (d).** Aplojaveediin A**; (e).** Alternatain D

albicans ATCC24433 strain and moderate antibacterial activity against the pathogenic bacterium *Staphylococcus aureus* (Gao et al. 2020). Four new polyketides compound namely alternatain A-D were extracted from the culture of *Alternaria alternata* MT-47, associated with the medicinal plant *Huperzia serrata*. Alternatain D (Fig. 5e) was reported to inhibit the thrombin activated platelets ATP release with an IC_{50} value of 57.6 μM and exhibited weak anti-platelet effects (Yang et al. 2019a).

Depsidones

Depsidones are polyketides that have been frequently isolated from lichens and widespread in Ascomycota (Elix et al. 1990). Depsidones have two rings of 2, 4-dihydroxybenzoic acid linked together by ester and ether bonds arising from polyketide biosynthesis. Depsidones exhibit various biological properties such as cytotoxic activity, antimalarial activity, antioxidant activity and anti-inflammatory activity (Ibrahim et al. 2018). Four novel compounds Mollicellins O-R and three known compounds Mollicellins G-I were isolated from the culture extracts of *Chaetomium* sp. Eef-10 associated with *Eucalyptus exserta*. Among the compounds extracted, mollicellin G (Fig. 6a) showed cytotoxic activity against two cancer cell line HepG2 and HeLa with weak IC_{50} value of 19.64 μg/mL and 13.97 μg/mL, respectively. Mollicellin H (Fig. 6b) showed best antibacterial activity against *Staphylococcus aureus* TCC29213 and *Staphylococcus aureus* N50 with IC_{50} value of 5.14 μg/mL and 6.21 μg/mL, respectively. Mollicellin O (Fig. 6c) exhibited antioxidant activity with an IC_{50} value of 71.92 μg/mL (Ouyang et al. 2018). Phomopsidone A (Fig. 6d), a pentacyclic depsidone, was extracted from the fungal strain *Diaporthe* sp. (originally reported as *Phomopsis* sp.), associated with the mangrove plant of China *Kandelia candel*. Phomopsidone A exhibited weak cytotoxic effect against MDA-MB-435 cell line with an IC_{50} value of 63 μM (Zhang et al. 2014). Guanxidone A (Fig. 6e), a tetracyclic depsidone derivative, was isolated from the *Aspergillus* sp. GXNU-A9 associated with the leaves of *Acanthus ilicifolius*. It showed anti-inflammatory activity and also significantly minimizes the production of nitric oxide in lipopolysaccharide stimulated cells with an IC_{50} value of 8.22 μM (Hao et al. 2020).

Lactones

Lactones are cyclic esters of polyketide origin produced by the lactonization (cyclization) of the hydroxyl acids. Lactones can be made through different pathways like β oxidation; ω-oxidation (Krzyczkowska et al. 2017). Brefeldin A (Fig. 7a), was isolated from various strains of the genera *Alternaria, Ascochyta, Cercospora, Penicillium,* and *Phyllosticta*. Brefeldin A was also reported to be produced from the endophytic fungi *Aspergillus clavatus* and *Paecilomyces* sp., associated with *Torreya grandis* and *Taxus mairei*, respectively. Brefeldin A showed antiviral, antifungal activity and also shows cytotoxic activity against Hela, HL-60 and MCF-7 cancer cells (Wang et al. 2002). From the fermentation broth of *Cladosporium* sp., Brefeldin A was extracted. *Cladosporium* sp. was isolated from the plant *Quercus variabilis* and exhibited antimicrobial activity (Wang et al. 2007a). Lactones such as eutypellin A (Fig. 7b) and eutypellin B (Fig. 7c) were extracted from an endophytic *Eutypella* sp. associated with *Etlingera littoralis* and showed cytotoxic activity (Isaka et al. 2009). A new lactone derivative hydroxylasiodiplodin (Fig. 7d) was extracted from the fungal strain M65 associated with *Azadirachta indica* (Neem). Hydroxylasiodiplodin was reported to inhibit the motility of zoospore of late blight phytopathogen *Phytophthora capsici* by 100% at 10 μg/mL concentration (Mondol et al. 2016). Penicimenolides A-F, six resorcylic acid lactone derivatives are isolated from the fungal broth of *Penicillium* sp. (SYP-F-7919) fungal strain associated with *Panax notoginseng* rhizosphere soil from China. Penicimenolides B-D (Fig. 7e–g) exhibited cytotoxic activity against MCF-7 and U937 cancer cell lines while moderate activity against SW480 and SH-SY5Y cell lines. The penicimenolides B-D showed inhibitory effect on nitric oxide production with an IC_{50} values ranging from 0.7 to 5.8 μM. By inhibiting nitric oxide production, penicimenolides B-D could potentially be helpful in the treatment of the diseases like inflammation and malignant tumors (An et al. 2016). The β-lactone polonicin A (Fig. 7h) the corresponding enoic acid polonicin B (Fig. 7i), two new compounds along with seven known compounds were extracted from the *Penicillium polonicum* associated with *C. acuminata*. Polonicin A exhibited GLUT4 translocation activities and significant glucose uptake in myoblast cell line of rat skeleton compared to control (Wen et al. 2020).

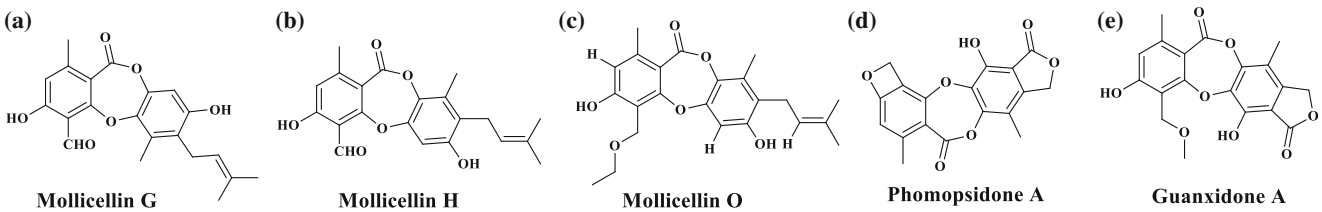

Fig. 6 Chemical structure of depsidones. **(a).** Mollicellin G**; (b).** Mollicellin H**; (c).** Mollicellin O**; (d).** Phomopsidone A**; (e).** Guanxidone A

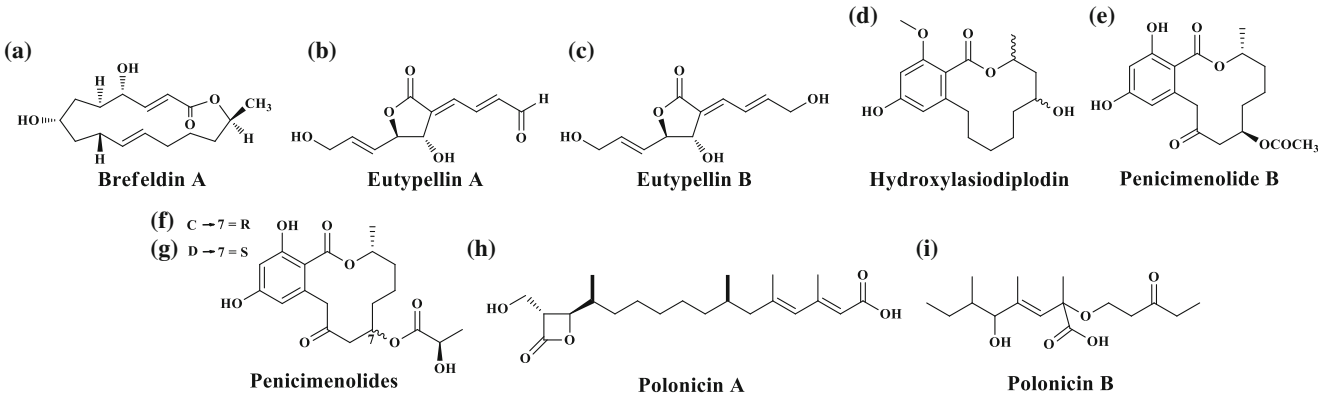

Fig. 7 Chemical structure of lactones. **(a).** Brefeldin A**; (b).** Eutypellin A**; (c).** Eutypellin B**; (d).** Hydroxylasiodiplodin**; (e).** Penicimenolide B**; (f).** Penicimenolide C**; (g).** Penicimenolide D**; (h).** Polonicin A**; (i).** Polonicin B

Chromones

Chromones are benzopyran derivatives of polyketide origin with a substituted keto group on the pyran ring. From the fermentation extract of *Diaporthe* sp. (originally reported as *Phomopsis* sp.), four new chromone derivatives named, phomopsichins A–D (Fig. 8a–d) were extracted. These compounds showed weak antimicrobial and antioxidant activity (Huang et al. 2016). Three new chromanones, phomochromanones A–C (Fig. 8e–g) were isolated from the culture of *Diaporthe* sp., associated with *Achyranthes bidentata*. Phomochromanones A–C were reported to induce apoptosis to the PANC-1 tumor cell line and also inhibit HIV-1 replication (Yang et al. 2020). Lawsozaheer (Fig. 8h), a new chromone compound, was extracted from the culture broth of an endophytic *Byssochlamys spectabilis* associated with *Lawsonia alba*. The broth extracts as well as lawsozaheer showed significant inhibitory activity against the bacterium *Staphylococcus aureus* (NCTC6571) at 150 μg/mL (Abbas et al. 2020). (2′*S*)-2-(2-Acetoxypropyl)-7-hydroxy-5-methylchromone (Fig. 8i), a new chromone derivative, was isolated from the ethyl acetate extract of an endophytic *Alternaria brassicae* JS959 associated with *Vitex rotundifolia* plant. (2′*S*)-2-(2-Acetoxypropyl)-7-hydroxy-5-methylchromone compound exhibited inhibitory activity against high density lipoprotein oxidation and copper induced low-density lipoprotein in human blood plasma and can be a potent molecule for treating heart ailments (Kim et al. 2019).

Quinones

Quinones are known to be present in various living organisms such as plants, humans and bacteria. Quinones and their conjugated structures are also known to be derived from fungi. However, only few biosynthetic pathways of quinone biosynthesis have been characterized so far. A study showed biosynthetic mechanism of quinone synthesis in *Beauveria* species via polyketide synthase pathways (Feng et al. 2015).

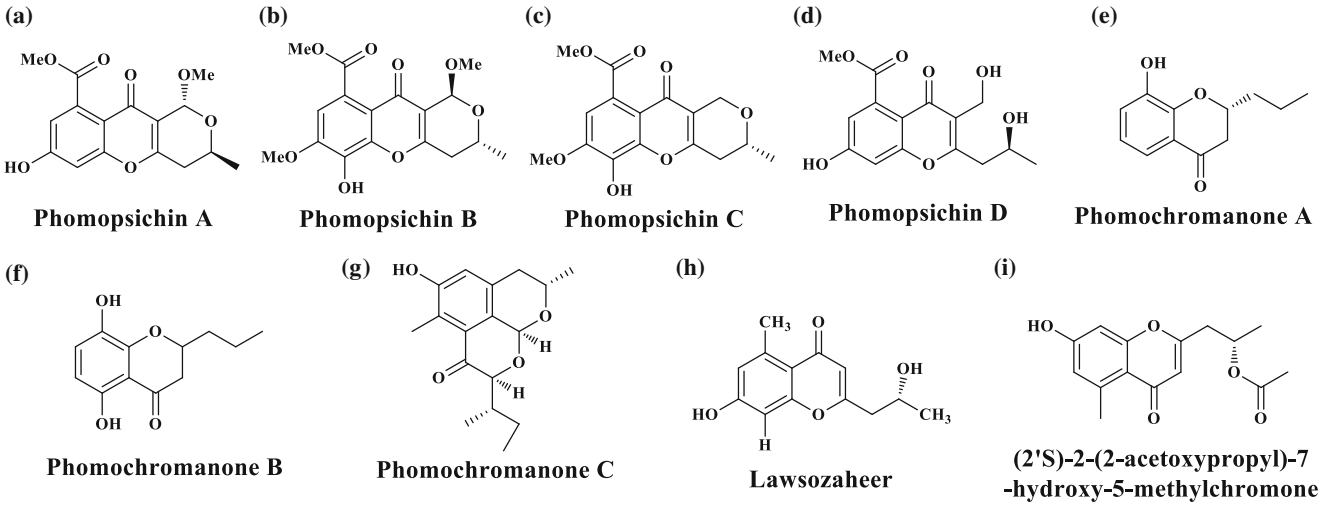

Fig. 8 Chemical structure of chromones. **(a).** Phomopsichin A**; (b).** Phomopsichin B**; (c).** Phomopsichin C**; (d).** Phomopsichin D**; (e).** Phomochromanone A**; (f).** Phomochromanone B**; (g).** Phomochromanone C**; (h).** Lawsozaheer**; (i).** (2′S)-2-(2-acetoxypropyl)-7-hydroxy-5-methylchromone**; Me:** CH_3.

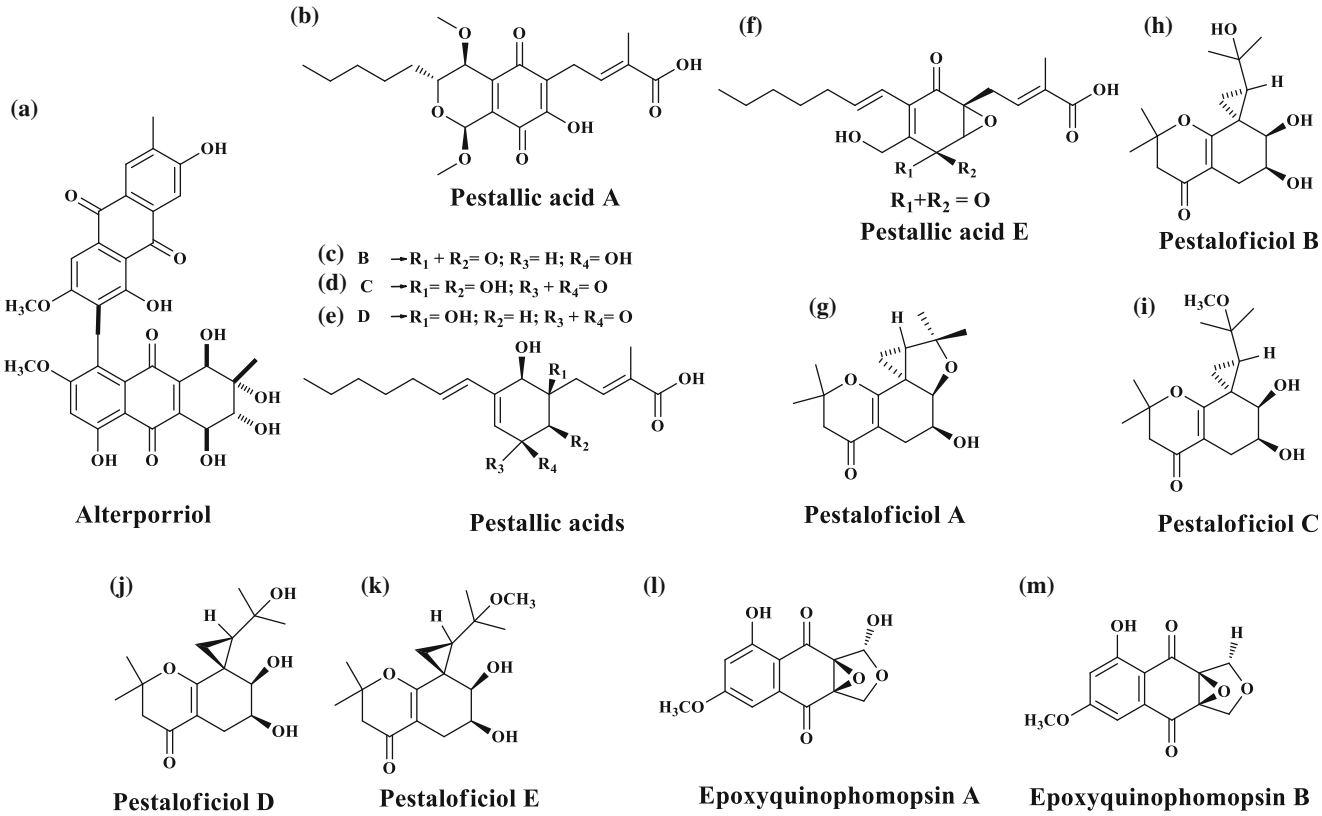

Fig. 9 Chemical structure of quinones. **(a).** Alterporriol; **(b).** Pestallic acid A; **(c).** Pestallic acid B; **(d).** Pestallic acid C; **(e).** Pestallic acid D; **(f).** Pestallic acid E; **(g).** Pestaloficiol A; **(h).** Pestaloficiol B; **(i).** Pestaloficiol C; **(j).** Pestaloficiol D; **(k).** Pestaloficiol E; **(l).** Epoxyquinophomopsin A; **(m).** Epoxyquinophomopsin B

Quinones are a class of bioactive compounds that are derivatives of aromatic compound such as benzene or naphthalene. Alterporriol (Fig. 9a), an anthanoid compound, showed cytotoxic activity against the L5178Y cancer cell line, with an EC_{50} value of 2.7 μg/mL, which was extracted from the rice culture of an endophytic *Stemphylium globuliferum* associated with the medicinal plant *Mentha pulegium* (Debbab et al. 2009). Pestallic acids A–E (Fig. 9b–f), an ambuic acid derivative, were extracted from the fungal endophyte *Pestalotiopsis* sp. FT172. Pestallic acid E showed cytotoxic activity against A2780 cancer cell line with an IC_{50} value of 3.3 μM (Li et al. 2017). Cyclopropane derivatives, named pestaloficiols A-E (Fig. 9g–k), were isolated from *Pestalotiopsis fici*. Pestaloficiols A, B, and D were reported to have inhibitory activity for HIV-1 replication in C8166 cells (Liu et al. 2008). The quinone derivatives, epoxyquinophomopsin A and B (Fig. 9l–m), were extracted from the endophyte *Diaporthe* sp. associated with *Morus cathayana*. Non-receptor tyrosine kinases were reported to inhibit by epoxyquinophomopsin A and B by 19% and 20%, respectively. Epoxyquinophomopsin A showed inhibitory activity against receptor tyrosine kinases EGFR (epidermal growth factor receptor) and HER-4 (human epidermal growth factor receptor 4) (Hermawati et al. 2020).

Therefore, based on the aforementioned examples, it can be concluded that fungal endophytes are a great source of potential bioactive metabolites. However, a great number of medicinal plants are still not screened for fungal endophytes to isolate potential bioactive metabolites which need to be explored in upcoming future.

Approaches for enhanced production of fungal metabolites

Some fungal metabolites which can be used in health care are produced in a low amount. In order to improve the yield of useful bioactive metabolites from the endophytes through industrial fermentation, various modifications are required. A combination of different methods can be applied including gene overexpression, optimization of culture parameter, precursor feeding, use of elicitors, cellular activity manipulation, and metabolic activity manipulation are an ongoing approach and can be used to enhance the production of novel metabolites (Morales-Sánchez et al. 2020).

Refinement of fungal strains

Fungal strain improvement not only improves the yield of the useful secondary metabolites but also removes unwanted

compounds, utilizes inexpensive nitrogen and carbon sources and in the transfer of oxygen in the fermentor by altering the cellular morphology. For commercial processing, the strain improvement method can be adopted, which can be further processed by microbial fermentation techniques. Using molecular biology and biochemical tools, strain improvement can be performed which may lead to the increased production of the associated bioactive metabolites.

Genetic engineering of endophytes for the production of metabolites can be done by overexpression of gene, genetic manipulation, or gene shuffling (Wang et al. 2016). Use of epigenetic modifiers for unlocking various gene clusters and inducing histone modification, DNA methylation through biotechnology approach also enhances the quality and quantity of the metabolites by modulating the metabolite mechanism (Poças-Fonseca et al. 2020). The genetic engineering for fungal endophyte is still at the preliminary level. Finding of a suitable transformation method and genetic manipulation of specific fungus is a challenging task, especially for endophytic fungi (Meyer 2008). Therefore, the foremost important thing is to design the most suitable transformation method. Various methods for the transformation of filamentous fungi include protoplasm mediated transformation, *Agrobacterium*-mediated transformation (Bernardi-Wenzel et al. 2016), electroporation method (Kumar et al. 2013), biolistic transformation (Poyedinok and Blume 2018), restricted enzyme-mediated transformation (Wang et al. 2007b), and lithium acetate transformation method (Wu and Letchworth 2004).

Optimization of culture parameters

A culture condition with right combination should be provided for a fungal endophyte to grow healthy and timely with maximum quantity from less substrate and to produce sufficient quality of bioactive metabolites. In a study, to determine the effect of different substrate with varying concentration response surface methodology was used (Gajdhane et al., 2016). Both single-factor optimization and statistical optimization have been studied in the fermentation process to produce the compounds which are not easily isolated (Li et al. 2016; Li et al. 2012b). The endophyte *Dichotomopilus funicola*, associated with the pigeon pea leaves, produces a glycosidic compound vitexin. This compound was produced using three culture media constituents such as salicylic acid, $CuSO_4 \cdot 5H_2O$, and L-phenylalanine. The vitexin yield under three constituents were 0.21g/L, 0.19g/L, and 0.06g/L, respectively which was 4.59-fold higher than in unoptimized culture medium (Gu et al. 2018). The metabolite production of the endophyte *Geosmithia pallida* (KU693285) associated with *Brucea mollis* was enhanced by growing it on modified media and by adding lactose and yeast extract along with NaCl (Deka and Jha 2018). However, the characterization and identification of bioactive metabolites were not done. Thus, by altering parameters of culture media, the secondary metabolites produced by fungal metabolites can be enhanced.

Co-culturing

Co-culturing is also a modified way to improve the secondary metabolite production. It consists of two or more endophytic fungi, which can activate the silent biosynthetic genes (do Nascimento et al. 2020). Sometimes it also initiates the production of metabolites from fungal endophytes (Akone et al. 2016). Ten citrinin analogs including seco-penicitrinol A and penicitrinol L were extracted by co-culturing of *Penicillium citrinum* EN-535 and *Aspergillus sydowii* EN-534. *Setophoma* sp., a fungal endophyte associated with the plant *Psidium guajava*, was co-cultured with *Penicillium brasilianum*. It led to the enhanced production of perylenequinones and antifungal compound stemphyperylenol (Bazioli et al. 2020). Co-culturing of endophytes could produce a specific bioactive metabolite that may or may not be produced by culturing of a single endophyte. Thus, co-culturing is an important approach for the production of different types of bioactive metabolites.

Precursor feeding

Precursor feeding is the method of providing the culture medium with biosynthetic derived precursor or its intermediates to increase the yield of desired compounds. Various attempts have been made to increase the production of metabolites for commercialization purposes in higher plants and now it has also been used for fungal endophytes (Wei et al., 2020). Indole-3-acetic acid is a phytohormone which is frequently produced by plant associated fungi as it easily arises from tryptophan. This compound was extracted by a fungal culture of *Colletotrichum fructicola* associated with the plant *Coffea arabica*. The compound yield was observed as 1205μg/mL which was highest as it was cultured in liquid medium supplemented with tryptophan (Numponsak et al. 2018). Curvulamine, a scaffold alkaloid with significant antibacterial activity, was produced by *Curvularia* sp. the marine fungal endophyte. The compound production was enhanced by 3.08-fold as compared to control by feeding the precursors alanine and proline (Wei et al., 2020). Adding the precursors in the media is known to enhance the production of bioactive metabolites as compared with the media with no adsorbent. Thus, it can be used for the enhancement of the bioactive metabolite production.

Conclusion and future perspectives

Medicinal plants are very important sources of wide range of bioactive metabolites which can be exploited as therapeutic agents against variety of disease (Kaul et al. 2012). Though several efforts have been made to study these valuable compounds and develop potent drugs for mankind, minimal success has been achieved to date. Despite enormous studies and vast literature against it, not a single new carbon skeleton with prominent activities has been discovered from them in the past 30 years that warrants development of new drug. Fungal endophytes are the symbionts of plants that appeared as emerging alternatives for new chemical diversity of compounds with beneficial effect on human health. In the present article, we have attempted to highlight the importance of bioactive metabolites isolated from fungal endophytes of medicinal plants. Fungal endophyte produces a wide range of structurally unique bioactive metabolites with various biological activities, making it useful for human health. To meet the demand of drug companies for commercialization, various biotechnological processes can be used including fermentation optimization, drug designing, genetic engineering, etc. Even after enormous advancement in the field of endophytic research, relationship between host-endophyte interaction and the extent of bioactive metabolite production is largely untapped. An in-depth analysis is still required to define the effect of environmental factors and host plants on the production of secondary metabolites by fungal endophytes to produce bioactive metabolites. Nowadays, keen interest of researchers is focused on the application of modern techniques such as in silico profiling of candidate genes, metabolomics, proteomic, transcriptomic, and genome mining for optimization and large-scale production of the bioactive metabolites. Modern techniques and advanced biotechnological interventions hold enormous prospects for the exploration and characterization of novel bioactive compounds derived from fungal endophytes in future.

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Code availability Not applicable

Author contribution PKK, NR, SCK, SB, PM, and SKS wrote and compiled the manuscript. PKK, NR, AV, and PS revised the manuscript under the supervision of VG. Study and entire writing of the manuscript was supervised by VG (from the compilation of the first draft to the final draft).

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Data Availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

Research involving human participants and/or animals The article does not include any human and/or animals-based study.

Informed consent Not applicable

References

Abbas Z, Siddiqui BS, Shahzad S, Sattar S, Begum S, Batool A, Choudhary MI (2020) Lawsozaheer, a new chromone produced by an endophytic fungus *Paecilomyces variotii* isolated from *Lawsonia alba* Lam. inhibits the growth of *Staphylococcus aureus*. Nat Prod Res 24:01–06

Adpressa DA, Loesgen SJC, Biodiversity (2016) Bioprospecting chemical diversity and bioactivity in a marine derived *Aspergillus terreus*. Chem Biodivers 13(2):253–259

Akone SH, Mándi A, Kurtán T, Hartmann R, Lin W, Daletos G, Proksch P (2016) Inducing secondary metabolite production by the endophytic fungus *Chaetomium* sp. through fungal–bacterial co-culture and epigenetic modification. Tetrahedron 72(41):6340–6347

Albright JC, Henke MT, Soukup AA, McClure RA, Thomson RJ, Keller NP, Kelleher NL (2015) Large-scale metabolomics reveals a complex response of *Aspergillus nidulans* to epigenetic perturbation. ACS Chem Biol 10(6):1535–1541

An Y-N, Zhang X, Zhang T-Y, Zhang M-Y, Qian Z, Deng X-Y, Zhao F, Zhu L-J, Wang G, Zhang J, Zhang Y-X, Liu B, Yao X-S (2016) Penicimenolides A-F, resorcylic acid lactones from *Penicillium* sp., isolated from the rhizosphere soil of *Panax notoginseng*. Sci Rep 6(1):27396

Ancheeva E, Daletos G, Proksch P (2020) Bioactive secondary metabolites from endophytic fungi. Curr Med Chem 27(11):1836–1854

Arora D, Sharma N, Singamaneni V, Sharma V, Kushwaha M, Abrol V, Guru S, Sharma S, Gupta AP, Bhushan S (2016) Isolation and characterization of bioactive metabolites from *Xylaria psidii*, an endophytic fungus of the medicinal plant *Aegle marmelos* and their role in mitochondrial dependent apoptosis against pancreatic cancer cells. Phytomedicine 23(12):1312–1320

Azie N, Angulo D, Dehn B, Sobel JD (2020) Oral Ibrexafungerp: an investigational agent for the treatment of vulvovaginal candidiasis. Expert Opin Investig Drugs 29(9):893–900

Backman PA, Sikora RA (2008) Endophytes: an emerging tool for biological control. Biol Control 46(1):01–03

Balagurunathan R, Radhakrishnan M, Shanmugasundaram T, Gopikrishnan V, Jerrine J (2020) Bioassay-Guided isolation and characterization of metabolites from Actinobacteria. Protocols in Actinobacterial Research. Springer, Berlin, pp 147–163

Bazioli JM, Fill TP, Rocha MC, Malavazi I, Filho ER, de Medeiros LS (2020) Perylenequinones production induced by co-culturing *Setophoma* sp. and *Penicillium brasilianum*. Phytochem Lett 40: 76–83

Bernardi-Wenzel J, Quecine M, Azevedo J, Pamphile J (2016) *Agrobacterium*-mediated transformation of *Fusarium proliferatum*. Genet Mol Res 15(2):01–12

Bilal S, Ali L, Khan AL, Shahzad R, Asaf S, Imran M, Kang S-M, Kim S-K, Lee I-J (2018) Endophytic fungus *Paecilomyces formosus* LHL10 produces sester-terpenoid YW3548 and cyclic peptide that inhibit urease and α-glucosidase enzyme activities. Arch Microbiol 200(10):1493–1502

Bills GF, Gloer JB (2016) Biologically active secondary metabolites from the fungi. Microbiol Spectr 4(6):1087–1119

Bills GF, González-Menéndez V, Martín J, Platas G, Fournier J, Peršoh D, Stadler M (2012) *Hypoxylon pulicicidum* sp. nov. (Ascomycota, Xylariales), a pantropical insecticide-producing endophyte. PLoS One 7(10):e46687

Blessie EJ, Wruck W, Abbey BA, Ncube A, Graffmann N, Amarh V, Arthur PK, Adjaye J (2020) Transcriptomic analysis of marine endophytic fungi extract identifies highly enriched anti-fungal fractions targeting cancer pathways in HepG2 cell lines. BMC Genomics 21:01–17

Bode HB, Bethe B, Höfs R, Zeeck A (2002) Big effects from small changes: possible ways to explore nature's chemical diversity. ChemBioChem 3(7):619–627

Bogner CW, Kamdem RS, Sichtermann G, Matthäus C, Hölscher D, Popp J, Proksch P, Grundler FM, Schouten A (2017) Bioactive secondary metabolites with multiple activities from a fungal endophyte. Microb Biotechnol 10(1):175–188

Brader G, Compant S, Vescio K, Mitter B, Trognitz F, Ma L-J, Sessitsch A (2017) Ecology and genomic insights into plant-pathogenic and plant-nonpathogenic endophytes. Annu Rev Phytopathol 55:61–83

Bunyapaiboonsri T, Yoiprommarat S, Srikitikulchai P, Srichomthong K, Lumyong S (2010) Oblongolides from the endophytic fungus *Phomopsis* sp. BCC 9789. J Nat Prod 73(1):55–59

Cadet R, Tufa M, Angulo D, Nyirjesy P (2019) A Phase 2b, dose-finding study evaluating oral ibrexafungerp vs fluconazole in vulvovaginal candidiasis (DOVE). Obstet Gynecol 133:113S–114S

Calcul L, Waterman C, Ma WS, Lebar MD, Harter C, Mutka T, Morton L, Maignan P, Olphen AV, Kyle DE (2013) Screening mangrove endophytic fungi for antimalarial natural products. Mar Drugs 11(12):5036–5050

Chapla VM, Zeraik ML, Leptokarydis IH, Silva GH, Bolzani VS, Young MCM, Pfenning LH, Araújo AR (2014) Antifungal compounds produced by *Colletotrichum gloeosporioides*, an endophytic fungus from *Michelia champaca*. Molecules 19(11):19243–19252

Chen L, Zhang Q-Y, Jia M, Ming Q-L, Yue W, Rahman K, Qin L-P, Han T (2016) Endophytic fungi with antitumor activities: their occurrence and anticancer compounds. Crit Rev Microbiol 42(3):454–473

Chen Y, Liu Z, Huang Y, Liu L, He J, Wang L, Yuan J, She Z (2019) Ascomylactams A–C, cytotoxic 12- or 13-membered-ring macrocyclic alkaloids isolated from the mangrove endophytic fungus *Didymella* sp. CYSK-4, and structure revisions of Phomapyrrolidones A and C. J Nat Prod 82(7):1752–1758

Chepkirui C, Stadler M (2017) The genus *Diaporthe*: a rich source of diverse and bioactive metabolites. Mycol Prog 16(5):477–494

Davis M, Donnelley M, Thompson G III (2020) Ibrexafungerp: a novel oral glucan synthase inhibitor. Med Mycol 58(5):579–592

Debbab A, Aly AH, Edrada-Ebel R, Wray V, Müller WE, Totzke F, Zirrgiebel U, Schachtele C, Kubbutat MH, Lin WH (2009) Bioactive metabolites from the endophytic fungus *Stemphylium globuliferum* isolated from *Mentha pulegium*. J Nat Prod 72(4): 626–631

Deka D, Jha DK (2018) Optimization of culture parameters for improved production of bioactive metabolite by endophytic *Geosmithia pallida* (KU693285) isolated from *Brucea mollis* Wall ex. Kurz, an endangered medicinal plant. J Pure Appl Microbio 12(3):1205–1213

Diaz PL, Hennell JR, Sucher NJ (2012) Genomic DNA extraction and barcoding of endophytic fungi. In: HJ SN, Carles M (eds) Plant DNA fingerprinting and barcoding, vol 862. Springer, Humana Press, pp 171–179

do Nascimento JS, Silva FM, Magallanes-Noguera CA, Kurina-Sanz M, dos Santos EG, Caldas IS, JHH L, EdO S (2020) Natural trypanocidal product produced by endophytic fungi through co-culturing. Folia Microbiol 65(2):323–328

Ekanayake PN, Rabinovich M, Guthridge KM, Spangenberg GC, Forster JW, Sawbridge TI (2013) Phylogenomics of fescue grass-derived fungal endophytes based on selected nuclear genes and the mitochondrial gene complement. BMC Evol Biol 13(1):01–15

Elix J, Jenkins G, Venables D (1990) New chlorine-containing Depsidones from lichens. Austr J Chem 43(1):197–201

Feng P, Shang Y, Cen K, Wang C (2015) Fungal biosynthesis of the bibenzoquinone oosporein to evade insect immunity. Proc Natl Acad Sci 112:11365–11370

Gajdhane SB, Bhagwat PK, Dandge PB (2016) Response surface methodology-based optimization of production media and purification of α-galactosidase in solid-state fermentation by *Fusarium moniliforme* NCIM 1099. 3. Biotech 6(2):01–14

Gao N, Shang Z-C, Yu P, Luo J, Jian K-L, Kong L-Y, Yang M-H (2017) Alkaloids from the endophytic fungus *Penicillium brefeldianum* and their cytotoxic activities. Chin Chem Lett 28(6):1194–1199

Gao Y, Wang L, Kalscheuer R, Liu Z, Proksch P (2020) Antifungal polyketide derivatives from the endophytic fungus *Aplosporella javeedii*. Bioorg Med Chem 28(10):115456

Gautam V, Sarkar AK (2015) Laser assisted microdissection, an efficient technique to understand tissue specific gene expression patterns and functional genomics in plants. Mol Biotechnol 57(4):299–308

Gautam V, Singh A, Singh S, Sarkar AK (2016) An efficient LCM-based method for tissue specific expression analysis of genes and miRNAs. Sci Rep 6:21577

Gianoulis TA, Griffin MA, Spakowicz DJ, Dunican BF, Sboner A, Sismour AM, Kodira C, Egholm M, Church GM, Gerstein MB (2012) Genomic analysis of the hydrocarbon-producing, cellulolytic, endophytic fungus *Ascocoryne sarcoides*. PLoS Genet 8(3):01–12

González-Menéndez V, Pérez-Bonilla M, Pérez-Victoria I, Martín J, Muñoz F, Reyes F, Tormo JR, Genilloud O (2016) Multicomponent analysis of the differential induction of secondary metabolite profiles in fungal endophytes. Molecules 21(2):234

Granados GM, McTaggart AR, Rodas CA, Roux J, Wingfield MJ (2020) Species of Cryphonectriaceae occupy an endophytic niche in the Melastomataceae and are putative latent pathogens of *Eucalyptus*. Eur J Plant Pathol 156(1):273–283

Gu CB, Ma H, Ning WJ, Niu LL, Han HY, Yuan XH, Fu YJ (2018) Characterization, culture medium optimization and antioxidant activity of an endophytic vitexin-producing *fungus Dichotomopilus funicola* Y3 from pigeon pea [*Cajanus cajan* (L.) Millsp.]. J Appl Microbiol 125(4):1054–1065

Hamzah TNT, Lee SY, Hidayat A, Terhem R, Faridah-Hanum I, Mohamed R (2018) Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, *Rhizophora mucronata*, and identification of potential antagonists against the soil-borne fungus, *Fusarium solani*. Front Microbiol 9:1707

Hao L, Zhou D, Qin X, Zhang W, Yang R, Li J, Huang X (2020) A new depsidone derivative from mangrove endophytic fungus *Aspergillus* sp. GXNU-A9. Nat Prod Res 27:01–05

Hawksworth DL, Lücking R (2017) Fungal diversity revisited: 2.2 to 3.8 million species. In: The Fungal Kingdom. American Society of Microbiology, pp 79–95

Helaly SE, Thongbai B, Stadler M (2018) Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascomycete order Xylariales. Nat Prod Rep 35(9):992–1014

Hermawati E, Ellita SD, Juliawaty LD, Hakim EH, Syah YM, Ishikawa H (2020) Epoxyquinophomopsins A and B from endophytic fungus *Phomopsis* sp. and their activity against tyrosine kinase. J Nat Med 75:01–06

Hiragun T, Peng Z, Beaven MA (2005) Dexamethasone up-regulates the inhibitory adaptor protein Dok-1 and suppresses downstream activation of the mitogen-activated protein kinase pathway in antigen-stimulated RBL-2H3 mast cells. Mol Pharmacol 67(3):598–603

Huang M, Li J, Liu L, Yin S, Wang J, Lin Y (2016) Phomopsichin A–D; four new chromone derivatives from mangrove endophytic fungus *Phomopsis* sp. 33. Mar Drugs 14(11):01–11

Ibrahim SRM, Mohamed GA, Al Haidari RA, El-Kholy AA, Zayed MF, Khayat MT (2018) Biologically active fungal depsidones: chemistry, biosynthesis, structural characterization, and bioactivities. Fitoterapia 129:317–365

Isaka M, Palasarn S, Lapanun S, Chanthaket R, Boonyuen N, Lumyong S (2009) γ-Lactones and ent-eudesmane sesquiterpenes from the endophytic fungus *Eutypella* sp. BCC 13199. J Nat Prod 72(9):1720–1722

Kaaniche F, Hamed A, Abdel-Razek AS, Wibberg D, Abdissa N, El Euch IZ, Allouche N, Mellouli L, Shaaban M, Sewald N (2019) Bioactive secondary metabolites from new endophytic fungus Curvularia. sp isolated from Rauwolfia macrophylla. PLoS One 14(6):e0217627

Kaul S, Gupta S, Ahmed M, Dhar MK (2012) Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. Phytochem Rev 11(4):487–505

Kerk NM, Ceserani T, Tausta SL, Sussex IM, Nelson TM (2003) Laser capture microdissection of cells from plant tissues. Plant Physiol 132(1):27–35

Khaldi N, Seifuddin FT, Turner G, Haft D, Nierman WC, Wolfe KH, Fedorova ND (2010) SMURF: genomic mapping of fungal secondary metabolite clusters. Fungal Genet Biol 47(9):736–741

Khan R, Naqvi STQ, Fatima N, Muhammad SA (2019) 30. Study of antidiabetic activities of endophytic fungi isolated from plants. Pure Appl Bio 8(2):1287–1295

Kim JA, Jeon J, Kim K-T, Choi G, Park S-Y, Lee H-J, Shim S-H, Lee Y-H, Kim S (2017) Draft genome sequence of an endophytic fungus, *Gaeumannomyces* sp. strain JS-464, isolated from a reed plant, *Phragmites communis*. Genome Announc 5(31):e00734–e00717

Kim JW, Kim J-Y, Li W, Ryu JY, Kim S, Shim SH (2019) Chromones with lipoprotein oxidation inhibitory activity from an endophytic fungus *Alternaria brassicae* JS959 derived from *Vitex rotundifolia*. J Antibiot 72(9):709–713

Krzyczkowska J, Phan-Thi H, Waché Y (2017) Lactone formation in yeast and fungi. In: Mérillon J-M, Ramawat KG (eds) Fungal metabolites. Springer International Publishing, Cham, pp 461–498

Kumar M, Sharma R, Dua M, Tuteja N, Johri AK (2013) Electrotransformation transformation system for root endophytic fungus *Piriformospora indica*. In: Varma A, Kost G, Oelmüller R (eds) *Piriformospora indica*. Soil Biology, vol 33. Springer, Berlin, pp 309–321

Larkin E, Hager C, Chandra J, Mukherjee PK, Retuerto M, Salem I, Long L, Isham N, Kovanda L, Borroto-Esoda K (2017) The emerging pathogen *Candida auris*: growth phenotype, virulence factors, activity of antifungals, and effect of SCY-078, a novel glucan synthesis inhibitor, on growth morphology and biofilm formation. Antimicrob Agents Chemother 61(5):e02396

Lenta B, Ngatchou J, Frese M, Ladoh Yemeda CF, Voundi S, Nardella F, Michalek C, Wibberg D, Silvere N, Tsamo E, Kaiser M, Kalinowski J, Sewald N (2016) Purpureone, an antileishmanial ergochrome from the endophytic fungus *Purpureocillium lilacinum*. ZNB: J Chem Sci 71(11):1159–1167

Li B, Tang M, Tang K, Zhao L, Guo S (2012a) Screening for differentially expressed genes in *Anoectochilus roxburghii* (Orchidaceae) during symbiosis with the mycorrhizal fungus *Epulorhiza* sp. Sci China Life Sci 55(2):164–171

Li P, Xu L, Mou Y, Shan T, Mao Z, Lu S, Peng Y, Zhou L (2012b) Medium optimization for exopolysaccharide production in liquid culture of endophytic fungus *Berkleasmium* sp. Dzf12. Int J Mol Sci 13(9):11411–11426

Li Y, Guo S, Zhu H (2016) Statistical optimization of culture medium for production of exopolysaccharide from endophytic fungus *Bionectria ochroleuca* and its antitumor effect *in vitro*. EXCLI J 15:211

Li C-S, Yang B-J, Turkson J, Cao S (2017) Anti-proliferative ambuic acid derivatives from Hawaiian endophytic fungus *Pestalotiopsis* sp. FT172. Phytochemistry 140:77–82

Li Y, Kong D, Fu Y, Sussman MR, Wu H (2020) The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol Biochem 148:80–89

Liao G, Wu P, Xue J, Liu L, Li H, Wei X (2018) Asperimides A–D, anti-inflammatory aromatic butenolides from a tropical endophytic fungus *Aspergillus terreus*. Fitoterapia 131:50–54

Liu L, Tian R, Liu S, Chen X, Guo L, Che Y (2008) Pestaloficiols A–E, bioactive cyclopropane derivatives from the plant endophytic fungus *Pestalotiopsis fici*. Bioorg Med Chem 16(11):6021–6026

Liu J, Wang X, Pu H, Liu S, Kan J, Jin C (2017) Recent advances in endophytic exopolysaccharides: Production, structural characterization, physiological role and biological activity. Carbohydr Polym 157:1113–1124

Lücking R, Aime MC, Robbertse B, Miller AN, Ariyawansa HA, Aoki T, Cardinali G, Crous PW, Druzhinina IS, Geiser DM, Hawksworth DL, Hyde KD, Irinyi L, Jeewon R, Johnston PR, Kirk PM, Malosso E, May TW, Meyer W, Öpik M, Robert V, Stadler M, Thines M, Vu D, Yurkov AM, Zhang N, Schoch CL (2020) Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding? IMA Fungus 11:01–32

MacMillan J (2001) Occurrence of gibberellins in vascular plants, fungi, and bacteria. J Plant Growth Regul 20(4):387–442

Masi M, Maddau L, Linaldeddu BT, Scanu B, Evidente A, Cimmino A (2018) Bioactive metabolites from pathogenic and endophytic fungi of forest trees. Curr Med Chem 25(2):208–252

Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R (2011) antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res 39(suppl_2):339–346

Meyer V (2008) Genetic engineering of filamentous fungi—progress, obstacles and future trends. Biotechnol Adv 26(2):177–185

Mishra VK, Passari AK, Chandra P, Leo VV, Kumar B, Uthandi S, Thankappan S, Gupta VK, Singh BP (2017) Determination and production of antimicrobial compounds by *Aspergillus clavatonanicus* strain MJ31, an endophytic fungus from *Mirabilis jalapa* L. using UPLC-ESI-MS/MS and TD-GC-MS analysis. PLoS One 12(10):e0186234

Mondol MAM, Farthouse J, Islam MT, Schüffler A, Laatsch H (2016) A new lactone from *Chaetomium globosum* strain M65 that inhibits the motility of zoospores. Nat Prod Commun 11(12):1865–1868

Morales-Sánchez V, Fe Andrés M, Díaz CE, González-Coloma A (2020) Factors affecting the metabolite productions in endophytes: biotechnological approaches for production of metabolites. Curr Med Chem 27(11):1855–1873

Naveen B, Ommi NB, Mudiraj A, Mallikarjuna T, Babu PP, Nagarajan R (2017) Total synthesis of penicinoline E, marinamide, methyl marinamide and their antimalarial activity. ChemistrySelect 2(11):3256–3261

Nevalainen H, Kautto L, Te'o J (2014) Methods for isolation and cultivation of filamentous fungi. In: Paulsen IT, Holmes AJ (eds) Environmental Microbiology: Methods and Protocols, vol 1096. Springer Nature, Berlin, pp 03–16

Newman DJ, Cragg GM (2020) Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod 83(3):770–803

Numponsak T, Kumla J, Suwannarach N, Matsui K, Lumyong S (2018) Biosynthetic pathway and optimal conditions for the production of indole-3-acetic acid by an endophytic fungus, *Colletotrichum fructicola* CMU-A109. PLoS One 13(10):e0205070

Nunnally NS, Etienne KA, Angulo D, Lockhart SR, Berkow EL (2019) *In vitro* activity of ibrexafungerp, a novel glucan synthase inhibitor against *Candida glabrata* isolates with FKS mutations. Antimicrob Agents Chemother 63(11):e01692

Ondeyka JG, Helms GL, Hensens OD, Goetz MA, Zink DL, Tsipouras A, Shoop WL, Slayton L, Dombrowski AW, Polishook JD (1997) Nodulisporic acid A, a novel and potent insecticide from a *Nodulisporium* sp. isolation, structure determination, and chemical transformations. J Am Chem Soc 119(38):8809–8816

Ouyang J, Mao Z, Guo H, Xie Y, Cui Z, Sun J, Wu H, Wen X, Wang J, Shan T (2018) Mollicellins O–R, four new depsidones isolated from the endophytic fungus *Chaetomium* sp. Eef-10. Molecules 23(12): 01–11

Pan R, Bai X, Chen J, Zhang H, Wang H (2019) Exploring structural diversity of microbe secondary metabolites using OSMAC strategy: a literature review. Front Microbiol 10:01–20

Paranagama PA, Wijeratne EK, Burns AM, Marron MT, Gunatilaka MK, Arnold AE, Gunatilaka AL (2007) Heptaketides from *Corynespora* sp. inhabiting the cavern beard lichen, *Usnea cavernosa*: first report of metabolites of an endolichenic fungus. J Nat Prod 70(11):1700–1705

Peláez F, Cabello A, Platas G, Díez MT, del Val AG, Basilio A, Martán I, Vicente F, Bills GF, Giacobbe RA, Schwartz RE, Onishi JC, Meinz MS, Abruzzo GK, Flattery AM, Kong L, Kurtz MB (2000) The discovery of enfumafungin, a novel antifungal compound produced by an endophytic *Hormonema* species biological activity and taxonomy of the producing organisms. Syst Appl Microbiol 23(3):333–343

Pérez Hemphill CF, Daletos G, Liu Z, Lin W, Proksch P (2016) Polyketides from the mangrove-derived fungal endophyte *Pestalotiopsis clavispora*. Tetrahedron Lett 57(19):2078–2083

Pfaller MA, Messer SA, Motyl MR, Jones RN, Castanheira M (2013) *In vitro* activity of a new oral glucan synthase inhibitor (MK-3118) tested against *Aspergillus* spp. by CLSI and EUCAST broth microdilution methods. Antimicrob Agents Chemother 57(2): 1065–1068

Pfaller MA, Messer SA, Rhomberg PR, Borroto-Esoda K, Castanheira M (2017) Differential activity of the oral glucan synthase inhibitor SCY-078 against wild-type and echinocandin-resistant strains of *Candida* species. Antimicrob Agents Chemother 61(8)

Poças-Fonseca MJ, Cabral CG, Manfrão-Netto JHC (2020) Epigenetic manipulation of filamentous fungi for biotechnological applications: a systematic review. Biotechnol Lett 42(6):885–904

Potshangbam M, Devi SI, Sahoo D, Strobel GA (2017) Functional characterization of endophytic fungal community associated with *Oryza sativa* L. and *Zea mays* L. Front Microbiol 8:325

Poyedinok N, Blume YB (2018) Advances, problems, and prospects of genetic transformation of fungi. Cytol Genet 52(2):139–154

Priebe S, Linde J, Albrecht D, Guthke R, Brakhage AA (2011) FungiFun: a web-based application for functional categorization of fungal genes and proteins. Fungal Genet Biol 48(4):353–358

Promputtha I, Lumyong S, Dhanasekaran V, McKenzie EHC, Hyde KD, Jeewon R (2007) A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. Microb Ecol 53(4):579–590

Qi X, Li X, Zhao J, He N, Li Y, Zhang T, Wang S, Yu L, Xie Y (2019) GKK1032C, a new alkaloid compound from the endophytic fungus *Penicillium* sp. CPCC 400817 with activity against methicillin-resistant *S. aureus*. J Antibiot 72(4):237–240

Qin J-C, Zhang Y-M, Gao J-M, Bai M-S, Yang S-X, Laatsch H, Zhang A-L (2009) Bioactive metabolites produced by *Chaetomium globosum*, an endophytic fungus isolated from *Ginkgo biloba*. Bioorg Med Chem Lett 19(6):1572–1574

Rai N, Kumari Keshri P, Verma A, Kamble SC, Mishra P, Barik S, Kumar Singh S, Gautam V (2021) Plant associated fungal endophytes as a source of natural bioactive compounds. Mycology:01–21

Reis IMA, Ribeiro FPC, Almeida PRM, Costa LCB, Kamida HM, Uetanabaro APT, Branco A (2018) Characterization of the secondary metabolites from endophytic fungi *Nodulisporium* sp. isolated from the medicinal plant *Mikania laevigata* (Asteraceae) by reversed-phase high-performance liquid chromatography coupled with mass spectrometric multistage. Pharmacogn Mag 14(59):495

Roman M, Hernandez C, Blanco D, Obrychi G, Helou S, Angulo D (2017) SCY-078 Phase 2 study in moderate and severe vulvovaginal candidiasis (VVC). In: Twenty seventh European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria

Rukachaisirikul V, Sommart U, Phongpaichit S, Sakayaroj J, Kirtikara K (2008) Metabolites from the endophytic fungus *Phomopsis* sp. PSU-D15. Phytochemistry 69(3):783–787

Saikkonen K, Wäli P, Helander M, Faeth SH (2004) Evolution of endophyte–plant symbioses. Trends Plant Sci 9(6):275–280

Schafhauser T, Jahn L, Kirchner N, Kulik A, Flor L, Lang A, Caradec T, Fewer DP, Sivonen K, van Berkel WJ (2019) Antitumor astins originate from the fungal endophyte *Cyanodermella asteris* living within the medicinal plant *Aster tataricus*. Proc Natl Acad Sci 116(52): 26909–26917

Scherkenbeck J, Jeschke P, Harder A (2002) PF1022A and related cyclodepsipeptides-a novel class of anthelmintics. Curr Top Med Chem 2(7):759–777

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109(6): 661–686

Schulz B, Boyle C (2006) What are endophytes? In: Schulz BJE, Boyle CJC, Sieber TN (eds) Microbial root endophytes, Soil Biology, vol 9. Springer, Berlin, pp 01–13

Shahzad R, Waqas M, Khan AL, Asaf S, Khan MA, Kang S-M, Yun B-W, Lee I-J (2016) Seed-borne endophytic *Bacillus amyloliquefaciens* RWL-1 produces gibberellins and regulates endogenous phytohormones of *Oryza sativa*. Plant Physiol Biochem 106:236–243

Shao C-L, Wang C-Y, Gu Y-C, Wei M-Y, Pan J-H, Deng D-S, She Z-G, Lin Y-C (2010) Penicinoline, a new pyrrolyl 4-quinolinone alkaloid with an unprecedented ring system from an endophytic fungus *Penicillium* sp. Bioorg Med Chem Lett 20(11):3284–3286

Sharma D, Pramanik A, Agrawal PK (2016) Evaluation of bioactive secondary metabolites from endophytic fungus *Pestalotiopsis neglecta* BAB-5510 isolated from leaves of *Cupressus torulosa* D.Don. 3. Biotech 6(2):01–14

Shinozuka H, Hettiarachchige IK, Shinozuka M, Cogan NO, Spangenberg GC, Cocks BG, Forster JW, Sawbridge TI (2017) Horizontal transfer of a ß-1, 6-glucanase gene from an ancestral species of fungal endophyte to a cool-season grass host. Sci Rep 7(1):01–11

Shiono Y, Tsuchinari M, Shimanuki K, Miyajima T, Murayama T, Koseki T, Laatsch H, Funakoshi T, Takanami K, Suzuki K (2007) Fusaristatins A and B, two new cyclic lipopeptides from an endophytic *Fusarium* sp. J Antibiot 60(5):309–316

Shrivastava N, Jiang L, Li P, Sharma AK, Luo X, Wu S, Pandey R, Gao Q, Lou B (2018) Proteomic approach to understand the molecular physiology of symbiotic interaction between *Piriformospora indica* and *Brassica napus*. Sci Rep 8(1):01–13

Smith MM, Warren VA, Thomas BS, Brochu RM, Ertel EA, Rohrer S, Schaeffer J, Schmatz D, Petuch BR, Tang YS (2000) Nodulisporic acid opens insect glutamate-gated chloride channels: identification of a new high affinity modulator. Biochem 39:5543–5554

Smith SA, Tank DC, Boulanger L-A, Bascom-Slack CA, Eisenman K, Kingery D, Babbs B, Fenn K, Greene JS, Hann BD (2008) Bioactive endophytes warrant intensified exploration and conservation. PLoS One 3(8):e3052

Tan X, Zhang X, Yu M, Yu Y, Guo Z, Gong T, Niu S, Qin J, Zou Z, Ding G (2019) Sesquiterpenoids and mycotoxin swainsonine from the locoweed endophytic fungus *Alternaria oxytropis*. Phytochemistry 164:154–161

Tawfike AF, Abbott G, Young L, Edrada-Ebel R (2017) Metabolomic-guided isolation of bioactive natural products from *Curvularia* sp., an endophytic fungus of *Terminalia laxiflora*. Planta Med 84(3): 182–190

Toju H, Tanabe A, Ishii HS (2016) Ericaceous plant–fungus network in a harsh alpine–subalpine environment. Mol Ecol 25(13):3242–3257

VanderMolen KM, Raja HA, El-Elimat T, Oberlies NH (2013) Evaluation of culture media for the production of secondary metabolites in a natural products screening program. AMB Express 3(1): 71

Verma A, Johri B, Prakash A (2014) Antagonistic evaluation of bioactive metabolite from endophytic fungus, *Aspergillus flavipes* KF671231. J Mycol 2014:01–05

Verma S, Gautam V, Sarkar AK (2019) Improved laser capture microdissection (LCM)-based method for isolation of RNA, including miRNA and expression analysis in woody apple bud meristem. Planta 249:2015–2020

Verpoorte R, Contin A, Memelink J (2002) Biotechnology for the production of plant secondary metabolites. Phytochem Rev 1(1):13–25

Wagenaar MM, Clardy J (2001) Dicerandrols, new antibiotic and cytotoxic dimers produced by the fungus *Phomopsis longicolla* isolated from an endangered mint. J Nat Prod 64(8):1006–1009

Wang J, Huang Y, Fang M, Zhang Y, Zheng Z, Zhao Y, Su W (2002) Brefeldin A, a cytotoxin produced by *Paecilomyces* sp. and *Aspergillus clavatus* isolated from *Taxus mairei* and *Torreya grandis*. FEMS Immunol Med Microbiol 34(1):51–57

Wang Y, Guo L-D, Hyde KD (2005) Taxonomic placement of sterile morphotypes of endophytic fungi from *Pinus tabulaeformis* (Pinaceae) in northeast China based on rDNA sequences. Fungal Divers 20:235–260

Wang FW, Jiao RH, Cheng AB, Tan SH, Song YC (2007) Antimicrobial potentials of endophytic fungi residing in *Quercus variabilis* and brefeldin A obtained from *Cladosporium* sp. World J Microbiol Biotechnol 23(1):79–83

Wang Y, Guo B, Miao Z, Tang K (2007b) Transformation of taxol-producing endophytic fungi by restriction enzyme-mediated integration (REMI). FEMS Microbiol Lett 273(2):253–259

Wang X, Zhang X, Liu L, Xiang M, Wang W, Sun X, Che Y, Guo L, Liu G, Guo L (2015) Genomic and transcriptomic analysis of the endophytic fungus *Pestalotiopsis fici* reveals its lifestyle and high potential for synthesis of natural products. BMC Genomics 16(1):01–13

Wang M, Zhang W, Xu W, Shen Y, Du L (2016) Optimization of genome shuffling for high-yield production of the antitumor deacetylmycoepoxydiene in an endophytic fungus of mangrove plants. Appl Microbiol Biotechnol 100(17):7491–7498

Wang L, Han X, Zhu G, Wang Y, Chairoungdua A, Piyachaturawat P, Zhu W (2018) Polyketides from the endophytic fungus *Cladosporium* sp. isolated from the mangrove plant *Excoecaria agallocha*. Front Chem 6:01–09

Wang R, Clarke BB, Belanger FC (2019a) Transcriptome analysis of choke stroma and asymptomatic inflorescence tissues reveals changes in gene expression in both *Epichloë festucae* and its host plant *Festuca rubra* subsp. *rubra*. Microorganisms 7(11):01–25

Wang W-X, Zheng M-J, Li J, Feng T, Li Z-H, Huang R, Zheng Y-S, Sun H, Ai H-L, Liu J-K (2019b) Cytotoxic polyketides from endophytic fungus *Phoma bellidis* harbored in *Tricyrtis maculata*. Phytochem Lett 29:41–46

Wei X-C, Liu C-Q, Zhang R-Y, Zheng G-F, An F-L, Lu Y-H (2020) Improvement of curvulamine production by precursors co-addition strategy in liquid culture of marine-derived fungus *Curvularia* sp. IFB-Z10. Appl Biochem Biotechnol 190(1):73–89

Wen Y, Lv Y, Hao J, Chen H, Huang Y, Liu C, Huang H, Ma Y, Yang X (2020) Two new compounds of *Penicillium polonicum*, an endophytic fungus from *Camptotheca acuminata* Decne. Nat Prod Res 34(13):1879–1883

Wilson D (1995) Endophyte: The evolution of a term, and clarification of its use and definition. Oikos 73(2):274–276

Wittstein K, Cordsmeier A, Lambert C, Wendt L, Sir EB, Weber J, Wurzler N, Petrini LE, Stadler M (2020) Identification of *Rosellinia* species as producers of cyclodepsipeptide PF1022 A and resurrection of the genus *Dematophora* as inferred from polythetic taxonomy. Stud Mycol 96:01–16

Wu S, Letchworth GJ (2004) High efficiency transformation by electroporation of *Pichia pastoris* pretreated with lithium acetate and dithiothreitol. Biotechniques 36(1):152–154

Yadav M, Yadav A, Yadav JP (2014) In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from Eugenia jambolana Lam. *Asian Pac J Trop Med* 7:256–261

Yadava PK, Bhuyan SK, Bandyopadhyay P (2015) Extraction of proteins for two-dimensional gel electrophoresis and proteomic analysis from an endophytic fungus. *Protoc Exch* 10:01–10

Yang H, Qi B, Ding N, Jiang F, Jia F, Luo Y, Xu X, Wang L, Zhu Z, Liu X, Tu P, Shi S (2019a) Polyketides from *Alternaria alternata* MT-47, an endophytic fungus isolated from *Huperzia serrata*. Fitoterapia 137:104282

Yang Y, Liu X, Cai J, Chen Y, Li B, Guo Z, Huang G (2019b) Genomic characteristics and comparative genomics analysis of the endophytic fungus *Sarocladium brachiariae*. BMC Genomics 20(1):01–20

Yang Z, Wu K, Xu Y, Xia X, Wang X, Ge M, Shao L (2020) Three novel chromanones with biological activities from the endophytic fungus *Phomopsis* CGMCC No. 5416. J Antibiot 73(3):194–199

Zhang AH, Wang XQ, Han WB, Sun Y, Guo Y, Wu Q, Ge HM, Song YC, Ng SW, Xu Q (2013) Discovery of a new class of immunosuppressants from *Trichothecium roseum* co-inspired by cross-kingdom similarity in innate immunity and pharmacophore motif. Chem Asian J 8(12):3101–3107

Zhang W, Xu L, Yang L, Huang Y, Li S, Shen Y (2014) Phomopsidone A, a novel depsidone metabolite from the mangrove endophytic fungus *Phomopsis* sp. A123. Fitoterapia 96:146–151

Zheng Y, Chiang T-Y, Huang C-L, Gong X (2018) Highly diverse endophytes in roots of *Cycas bifida* (Cycadaceae), an ancient but endangered gymnosperm. J Microbiol 56(5):337–345

Zhou W, Wu Y, Chu L, Li W, Li H (2015) Endophytic fungal diversity of four bryophyte species in Dawei Mountain, Southwest of China. Wei Sheng Wu Xue Bao 55(6):764–771

Zhou P, Wu Z, Tan D, Yang J, Zhou Q, Zeng F, Zhang M, Bie Q, Chen C, Xue Y, Luo Z, Wang J, Zhu H, Zhang Y (2017) Atrichodermones A–C, three new secondary metabolites from the solid culture of an endophytic fungal strain, *Trichoderma atroviride*. Fitoterapia 123: 18–22