Chapter 12
Secondary Metabolites of Endophytic Fungi Against Candidiasis

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Abstract Plants have the ability to produce bioactive substances that aid in the treatment or prevention of disease. They also offer endophytes a special environment. The bulk of these endophytes are fungi that defend their hosts from infections through a mutualistic connection, yet occasionally they may also behave as opportunistic pathogens. Many endophytic fungi have been identified as sources of novel metabolites with potential for use in pharmaceuticals. Also, they have the capacity to produce a variety of bioactive metabolites that may be employed, either directly or indirectly, as therapeutic agents against a wide range of diseases and have drawn the interest worldwide due to their strong demand. Invasive fungal infections brought on by *Candida* species have emerged during the past three decades as a significant public health issue due to their high rates of morbidity and mortality in immunocompromised and hospitalized patients. Candida infections are difficult to diagnose and typically respond poorly to therapy. As a result, a variety of medicines that are now used to treat candidiasis commonly cause resistance in individuals, encouraging toxicity as a result of prolonged therapy. Therefore, a precise diagnostics and cutting-edge antifungals are of utmost importance in order to raise the quality of life and life expectancy of those affected with this infection. Several plant groups include a variety of bioactive secondary metabolites, including terpenoids, organosulfur compounds, isoquinoline alkaloids, flavonoids, fatty acids, lactone, naphthoquinone, phenolic compounds, etc. Numerous researchers have examined the inhibitory mechanisms of endophytic fungal bioactives against *Candida albicans* and other *Candida* spp.

Keywords Endophytes · Metabolites · Pharmaceuticals · Bioactive substances · Infections · Candidiasis

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12.1 Introduction

The phrases endophyte and endophytic fungus have been used extensively over the past 30 years to refer to the internal mycotic of living plants in the mycological literature. Notwithstanding the phrases' nineteenth-century roots, they now have a completely different meaning than when they were first coined (Large 1962; Larran et al. 2007). To describe a particular host type, a taxonomic group of hosts, or the type of tissue occupied, the phrases are frequently used in combination with modifiers (e.g., systemic grass endophytes, bark endophytes). The terms are not always used consistently today, and not all researchers agree with them (Pereira et al. 1993; Saikkonen et al. 1998). Yet, in general, the words refer to fungi that can asymptomatically occupy seemingly healthy plant tissue. Endophytic fungi, in the broadest sense, are fungi that invade living plant tissue without immediately manifesting any detrimental effects (Hirsch and Braun 1992). This definition essentially encompasses the full range of symbiotic relationships that fungi and plants engage in, including parasitism, commensalism, and mutualism.

Forest pathologists classify many of the fungi that are frequently described as endophytes as minor or secondary diseases. Their frequent appearances in both healthy and unhealthy tissues highlight how difficult it is to draw clear distinctions between endophytes, facultative pathogens, and latent pathogens. In fact, the behavioral differences between many fungi regarded as "endophytic" and those regarded as "latent pathogens" are negligible and may only be caused by variations in the length of the latent or quiescent phase and the severity of the host harm incurred during the fungus' active growth (Schardl et al. 1994).

Endophytes include a variety of commensal saprobic and mutualistic fungi that have cryptic, imperceptible patterns of host colonization, as well as pathogenic fungi that can occupy their hosts asymptomatically for part of the infection cycle, "quiescent infections," and strains with reduced virulence. This extended, unnoticeable time during which growth and colonization temporarily halt and then resume when the host undergoes a physical or maturational change is a hallmark of "endophytic" fungi. Whether endophytes are ultimately classified as commensal saprobes, latent pathogens, or protective mutualists, this periodic growth is a distinctive characteristic of them. The majority of fungal biologists agree that, despite the fact that such a definition might seem overly broad, the species composition of the internal mycobiota differs for different hosts, organs, and tissues (Fisher et al. 1992; Freeman and Rodriguez 1993). However, some endophytic infection species may also be present in the epiphytic or rhizosphere mycobiota.

As a result of recruitment, plants formed symbioses with a wide variety of soil microorganisms, some of which can live inside plant tissues and interact with the host endophytically. The endophytic fungi may first adhere to the surface of the roots and develop structures resembling appressoria (Yedidia et al. 1999). These associated fungi subsequently go into and populate the internal tissues of plants after penetrating the root systems' outer layers (Nogueira-Lopez et al. 2018; Viterbo and Chet 2006). The integrity of the cells was not compromised during the early

colonization of roots by the endophytic fungus *Trichoderma*, according to microscopic examinations. However, the necrosis of the penetration peg, the elevated chitinase activity, and the generation of fluorescent chemicals in the intercellular gaps were seen in cucumber roots that had been colonized by endophytes. This could be as a result of the extensive extracellular enzyme production by the endophytic fungus (Suryanarayanan et al. 2012). In actuality, rather than remaining static, the established endophytic interactions respond to real-time dynamic change. Citrus diseases, or the yellowing of the leaves, have been demonstrated in studies to influence foliar endophytic communities more than endophyte assemblages of healthy leaves. This shows that certain endophytes may grow more quickly when a leaf becomes yellow than others (Douanla-Meli et al. 2013). Microbial communities are influenced by an interaction between host genotype and abiotic conditions. By interactions between microbes, transmission of the impacts to the microbial community, and altering the architecture of plant microbial communities, these differences have a direct impact on a small number of closely related taxa and have a significant impact on communities (Agler et al. 2016). To counteract reduced plant photosynthesis and altered host nitrogen metabolism, endophytic fungus can alter the genetics and phenotypic expression of the host to increase resistance to diseases and herbivores (Mejía et al. 2014). Endophytic fungi retain symptomless survival and benefit their host plants thanks to this dynamic regulation. Hence, the precise control of host genes, phenotypes, and metabolism results in the relationship between endophytic fungi and their host plants.

Endophytes would create a variety of bioactive substances that would aid host plant growth while also assisting the host plants in coping with external biotic and abiotic challenges. Some endophytic fungi have the capacity to produce bioactive compounds that are identical or similar to those that come from the host plants. Paclitaxel, podophyllotoxin, camptothecin, vinblastine, hypericin, and diosgenin, which were also produced by their host plants, were created by the endophytic fungi. The applications of endophytic fungi in various fields are shown in Fig. 12.1.

The term "candidiasis" is used to refer broadly to cutaneous, mucosal, and deep-seated organ infections brought on by fungi of the *Candida* genus. These illnesses can strike at any age and are typically associated with readily observable risk factors for infection. A deep-seated infection such as an intra-abdominal abscess, peritonitis (inflammation of the peritoneum, the tissue covering the inner wall of the abdomen and abdominal organs), or osteomyelitis (infection of the bones), with or without candidemia, is referred to as invasive candidiasis (Chowdhary et al. 2017; Lockhart et al. 2017). A developing infection directly related to technological developments in medicine, invasive candidiasis is widely acknowledged as a leading source of morbidity and mortality in the medical setting (Clancy and Nguyen 2017). At least 15 different *Candida* species are capable of harming humans, although just five of them—*Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei*—are responsible for the bulk of invasive infections. *Candida auris*, a relatively uncommon microbe, has become a significant disease in several parts of the world (Magill et al. 2014; McCarty and Pappas 2016; Wisplinghoff et al. 2004).

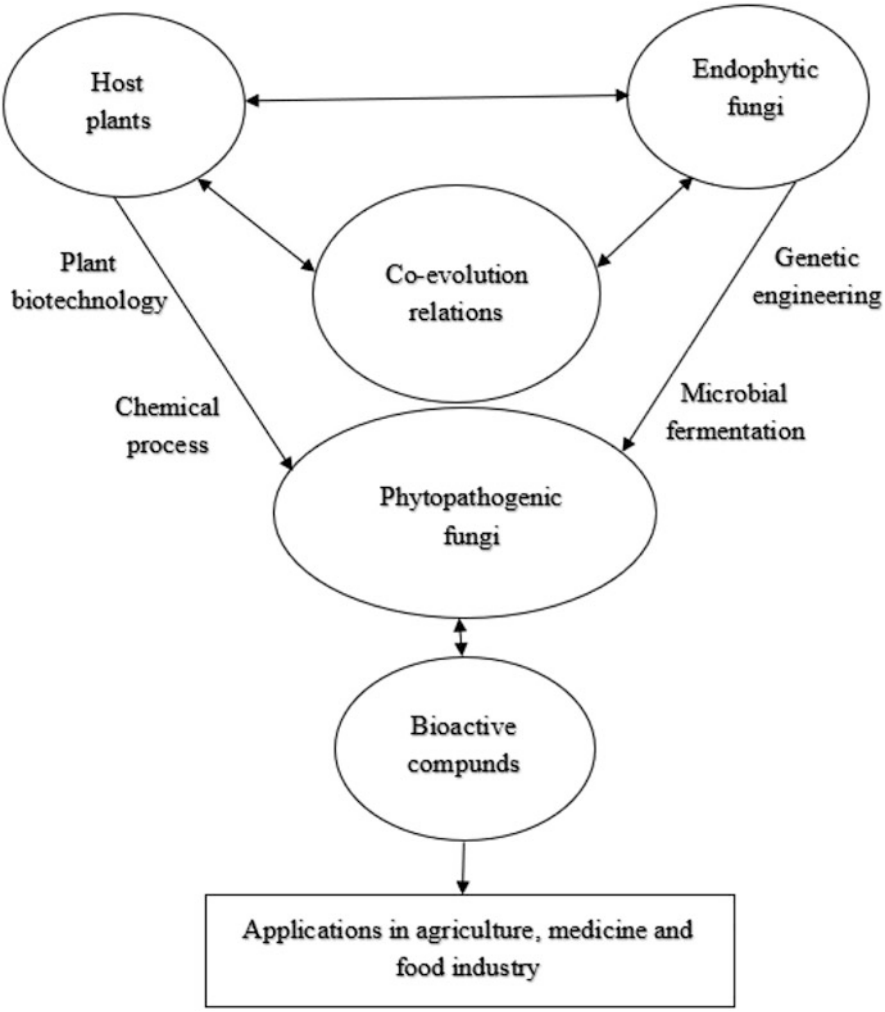

Fig. 12.1 Applications of endophytic fungi in various fields

Together with *Staphylococcus aureus*, coagulase-negative staphylococci, and *Enterococcus* species, *Candida* spp. are among the top three or four bacteria responsible for healthcare-associated bloodstream infections in many affluent nations. According to data from the National Healthcare and Safety Network, *Candida* spp. may be to blame for up to 22% of bloodstream infections connected to healthcare in the United States; it should be noted that this was a very selective patient cohort made up only of people using antibiotics (Magill et al. 2014). A point prevalence assessment of ICUs from throughout the world found that 18% of infections were caused by *Candida* spp. (Vincent et al. 2009; Playford et al.

2009). The complexity of sickness often linked to this infection is reflected by the fact that 50% of episodes of candidemia take place in the ICU. All patients in hospitals are at risk for developing invasive candidiasis, but those in the intensive care unit are more vulnerable due to well-described risk factors. Some risk factors originate from iatrogenic interventions, whereas others are inherent to the host or the disease state. Indwelling central venous catheters, broad-spectrum antibacterial exposure, prolonged ICU stays with or without assisted ventilation, recent major surgery, necrotizing pancreatitis, any form of dialysis, total parenteral nutrition, and iatrogenic immunosuppression are among the most prevalent individual risk factors (Wisplinghoff et al. 2004).

Age, local epidemiology, geographic location, and other variables all affect candidemia occurrence. Incidence rates of 3–5 per 100,000 people in the general population and 1–2% of all admissions to medical and surgical ICUs are reported in the majority of big national surveys (McCarty and Pappas 2016). A relatively recent discovery in the United States and other developed nations, community-acquired candidemia (i.e., acquired outside of a hospital) is a result of increased use of long-term intravenous access devices (such as peripherally inserted central catheters and tunneled intravascular catheters) and parenteral outpatient antimicrobial therapy. A more accurate estimate of the attributable mortality rate for all patients with candidemia is likely to be between 10 and 20%, with the risk of death being closely related to growing older, having higher APACHE II scores, having the infecting *Candida* species (e.g., *C. parapsilosis* is less virulent than other *Candida* species and is typically associated with lower all-cause mortality), and using immunosuppressive medications. According to reports, the attribute cost of candidemia is around US $40,000 per patient (Strollo et al. 2017).

12.2 Endophytic Fungal Diversity

The majority of endophytic fungi are *Ascomycota* members or their mitosporic fungus, together with a few species from *Basidiomycota*, *Zygomycota*, and *Oomycota* (Drew and Demain 1977). These endophytic fungi form symbiotic-to-pathogenic connections with the host while living inside the host's live tissues. These fungi colonize plants in a variety of ways, and some therapeutic plants are said to host more endophytes than others. It is recognized that the endophytes attached to medicinal plants have the capacity to manufacture the active ingredients for which the host is famous (Zaynab et al. 2019; Zheng and Jiang 1995; Perotto et al. 2002). Many endophytic fungi isolated from various groups of plants have been identified using both conventional taxonomic techniques and molecular taxonomy. It is well known that the isolated fungus creates a variety of chemicals that are both unique to the host and also universal to all hosts. In terms of characterization, species diversity, and bioactive compounds, research on the diversity of endophytic fungus is crucial (Huang et al. 2008; Verekar et al. 2014; Nadeem et al. 2012). Sequences of the 5.8S gene and the adjacent internal transcribed spacers (ITS1 and ITS2) of the rDNA,

18S, and 28S rRNA genes are utilized as molecular tools to research endophytic fungi. It is likely that many endophytic fungi cannot be cultivated due to the drawbacks of conventional isolation techniques (Garyali et al. 2013; Lucero et al. 2011). Molecular methods have also been used to identify endophytic fungi directly in the host tissues, overcoming any potential technical bias (Deckert et al. 2001).

12.3 Bioactive Metabolites of Endophytic Fungi

On our globe, there could be up to one million different types of fungi, according to estimates. Plant scientists have just started to understand that plants may act as a reservoir for countless numbers of endophytes or other microorganisms (Hawksworth and Rossman 1997). Some of these endophytes might be generating bioactive compounds that could have a role in the connection between the host and the endophyte. These metabolites may ultimately be found to be useful in medicine as a direct result of the role that they may play in nature. Endophytes are currently being isolated, and their natural products are being studied by scientists all over the world (Strobel 2003).

12.3.1 Primary Metabolites

Catabolic enzymes work in microbial cells to break down complex, high-molecular-weight carbon and energy sources as they expand. The finished goods of primary intermediates such amino acids, nucleotides, vitamins, carbohydrates, and fatty acids are produced as a result of catabolism. These biosynthetic intermediates are subsequently put together to form the intricate and crucial metabolites that give organisms their structure and biological function. The pathways that make up primary metabolism are made up of several mutually dependent catabolic and biosynthetic processes. The substances and enzymes of primary metabolism play fundamental, clearly defined, and frequently crucial roles in supporting microbial growth and reproduction (Drew and Demain 1977).

On the other hand, substances produced by secondary metabolism have hidden roles in the survival, growth, and reproduction of the generating organisms. Those substances and the specific enzymatic processes required for their synthesis seem to be of secondary importance to the organism and not necessary for growth. Although secondary metabolites differ from primary metabolites in terms of both structure and metabolic activity, this division tends to downplay the intricate interactions between primary and secondary metabolism. As the precursors for secondary metabolism are frequently provided by major metabolic pathways, we would anticipate that variables affecting primary metabolism would also affect secondary metabolism. It is clear that primary metabolism requires metabolic regulation. Efficiency plays a

significant role in microbial species survival, just like it does in all competitive processes.

A variety of microbial species can effectively compete in nature for the finite resources required for growth and reproduction due to the regulatory mechanisms that have developed to control the synthesis and function of the hundreds of enzymes involved in primary metabolism. Although our knowledge of these processes is fairly restricted, we may comment on some fundamental forms of regulatory activity. Primary metabolism is regulated by a number of different regulatory systems (Drew and Demain 1977; Zaynab et al. 2019).

When plants in a natural environment are exposed to a variety of diseases, they naturally create defense to preserve their fitness and reduce pathogenic harm. Research on the relationship between plants and diseases is becoming more important since they use primary metabolites as weapons. Pathogenesis and resistance are the ultimate outcomes of either defense strategy (Ferreira et al. 2007). Plant defense mechanisms can be divided into two categories: chemical, structural, and morphological defense and inducible and constitutive defense. A few defense mechanisms are quite active, but the majority are passive and only defensive against infections (Passardi et al. 2004). The significance of plant primary metabolites in immunity is attracting attention. Through signal transduction and pathogen identification mechanisms, primary metabolites carry out their role as molecules signaling to initiate a defense response. Primary metabolite production starts when the necessary nutrients are present in a growth medium during the active development phase (trophophase). Cell growth, development, and reproduction depend on primary metabolism (Penninckx et al. 1996). The primary metabolites participate in the primary response by controlling the synthesis of proteins, carbohydrates, and lipids in response to pathogen infection. Primary and secondary metabolism during pathogen infection affects plant growth and productivity. Supplying energy is necessary for the defense reaction (Gao et al. 2000). When there is a shortage of nutrients, pathogens can readily influence plant metabolism, which raises the requirement for nutrients to be assimilated. The reason metabolism slows down is that photosynthesis is reduced after a pathogen infection, which alters the growth of necrotic and chlorotic tissue and the sugar accumulation (Broekaert et al. 1995). There is very little information on the role of major metabolic pathways in the control of plant defense responses for growth and development (Kishimoto et al. 2002).

The host plant produces pathogenesis-related (PR) proteins, but these proteins are only activated in pathological conditions like bacterial, viral, or fungal diseases. The PR-9 family's peroxidase activity may contribute to cell wall fortification by catalyzing lignification, which would boost pathogen resistance. Families PR-12, PR-13, and PR-14 demonstrated antifungal properties. Proteins PR-15 and PR-16 produce hydrogen peroxide, which may be useful for enhancing plant defense or detrimental to intruders (Yamamoto et al. 2000). Two defensins (RsAFP1 and RsAFP2) were produced by *Alternaria brassicicola*-inoculated leaves. When pathogens attack, defensin inducibility has been seen in tobacco and *Arabidopsis* sepals. Several investigations have shown that plant defensins shield vegetative tissue against pathogen attack (Takahashi et al. 2005). By increasing defensin constitutive

expression, tobacco and tomato plants are defended against *Alternaria longipes* and *Alternaria solani*, respectively, which are fungal diseases (Rafin et al. 2000). By constitutively expressing *Brassica napus* defensin, resistance to the *Leptosphaeria maculans*-caused blackleg disease is increased. The potato exhibited a higher resistance to *Verticillium dahliae* due to defensins' constitutive expression (Tomoya et al. 2013). *Rhizoctonia solani* was reduced in canola and tobacco transgenic plants by overexpressing PR-3. Carrot and *N. sylvestris* were more resistant to *R. solani* when treated with tobacco chitinase. *Magnaporthe grisea*, *R. solani*, *B. cinerea*, *Uncinula necator*, and *Puccinia coronata* were all susceptible to rice chitinases' efficacy. *Fusarium oxysporum* in vitro activity of carbamic esters was assessed (Majumdar et al. 2017). Thionins with antifungal qualities have the ability to cause the formation of open pores on phytopathogens' cell membranes, which leads to the release of calcium and potassium ions from the cell and the antifungal activity of the Thi2.4 protein against *F. graminearum*. Maize's ZmPRms protein increases resistance to *Aspergillus flavus* (Majumdar et al. 2017). *R. solani*-induced tomato foot rot and the expression of defense-related genes including chitinase and peroxidase in the resistant and susceptible cultivars were assessed. The important aspect of a plant's basic defense mechanisms is the creation of physical barriers at places of attempted fungal penetration. These structures stop pathogen growth in plant tissues (Taheri and Tarighi 2012).

12.3.2 Secondary Metabolites

Organic compounds known as secondary metabolites are not necessary for an organism's regular growth and development. While primary metabolites play a crucial role in the survival of the species by actively participating in photosynthesis and respiration, the absence of secondary metabolites results in long-term impairment of the organism's survivability instead, which frequently plays a crucial role in plant defense. These substances are a hugely varied category of organic materials produced by a wide range of organisms, including plants, fungi, bacteria, algae, and mammals. The majority of secondary metabolites, including terpenes, phenolic compounds, and alkaloids, are categorized according to where they were created synthetically. Several medicinal, aromatic, colorant, and spice plants as well as some functional foods contain various classes of these chemicals, which are frequently linked to a small number of species within a phylogenetic group.

A transition from active growth to stationary phase typically results in the highest amounts of secondary metabolite production. The fact that the producer organism can develop without them suggests that secondary metabolism is not necessary, at least for immediate survival. According to a different theory, the genes responsible for secondary metabolism act as a "genetic playing field" on which natural selection and mutation can work together to fix new advantageous features. According to a third viewpoint, secondary metabolism is an essential component of cellular metabolism and biology. It depends on primary metabolism for the enzymes, energy,

substrates, and cellular machinery that it needs to function, and it helps ensure the producer's long-term survival (Roze et al. 2011).

Terpenes (such as plant volatiles, cardiac glycosides, carotenoids, and sterols), phenolics (such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins, and lignins), and nitrogen-containing chemicals make up a straightforward classification of secondary metabolites (such as alkaloids and glucosinolates). It has been claimed that a number of conventional separation methods using different solvent systems and spray reagents can distinguish between secondary metabolites using diverse adsorbents and eluents through column chromatography (CC) and thin layer chromatography.

12.3.2.1 Biological Activities

Numerous endophytic fungi have been identified as sources of new metabolites with potential medicinal use. Additionally, they have the capacity to produce a variety of bioactive metabolites, which may be employed directly or indirectly as therapeutic agents against a wide range of diseases. Endophytes have a variety of bioactive compounds that can be used to make essential medicinal medications commercially (Zhang et al. 2006). These bioactive compounds are primarily secondary metabolites and have been shown to have a variety of pharmacological effects. Many beneficial bioactive substances with antibacterial, cytotoxic, anticancer, antioxidant, antimalarial, antiviral, and antituberculotic activity have been successfully isolated from the endophytic mycoflora over the past two decades. From the endophytic fungus *Phomopsis longicolla* S1B4 isolated from a plant in South Korea, Lim et al. extracted various antibacterial chemicals, including dicerandrol A (1), dicerandrol B (2), dicerandrol C (3), deacetylphomoxanthone B (4), and fusaristatin A (5). The minimum inhibitory concentrations (MICs) of these substances against *Xanthomonas oryzae* KACC 10331 were 8, 16, 4, and 128 g/mL, respectively. Dicerandrol A also demonstrated antibacterial action against *Bacillus subtilis* KCTC 1021 and *S. aureus* KCTC 1916. Sim et al. isolated 24 endophytic fungi from *Garcinia mangostana* and *Garcinia parvifolia* and used filtered broth suspension to examine the antibacterial activity (Lim et al. 2010). On average, eleven isolates (or 46%) had antibacterial activity against one or more test organisms. An antimicrobial action was displayed by *Colletotrichum* sp., which was isolated from the medicinal herb *Lippia sidoides*. From the leaves and stems of *L. sidoides*, a total of 203 endophytic fungi representing 14 species of *Ascomycota*, *Coelomycetes*, and *Hyphomycetes* were isolated. The most typical fungus that was isolated was *C. gloeosporioides*, which was followed by *Alternaria alternata*, *Guignardia bidwellii*, and *Phomopsis archeri*. *S. aureus* and *B. subtilis* were resistant to the endophytic fungus *A. alternata*, *P. archeri*, *C. gloeosporioides*, and *Drechslera dematioidea's* antimicrobial effects (Sim et al. 2010; Ichikawa et al. 1971). The medicinal plant *Michelia champaca* produces a variety of secondary metabolites with a wide range of pharmacological characteristics. This plant's endophytic fungus exhibits antifungal, anticancer, and acetylcholinesterase (AChE)-inhibiting

properties. The activity of each extract was seen against two phytopathogenic fungi. The endophytic fungus *C. gloeosporioides* ethanol extracts produced eight known compounds, including 2-phenylethyl 1H-indol-3-ylacetate, uracil, cyclo-(S*-Pro-S*-Tyr), cyclo-(S*-Pro-S*-Val), 2-(2-aminophenyl)acetic acid, 2-(4-hydroxyphenyl)acetic acid, 4-hydroxy-benzamide, and 2-(2-hydroxyphenyl) acetic acid (Chapla et al. 2014). Numerous strains of *Fusarium* spp. produce enniatins (ENs), six-membered cyclic depsipeptides, as secondary metabolites. Meca et al. used reverse-phase low-pressure liquid chromatography (LPLC) on Amberlite XAD-7 to extract ENs A, A1, B, and B1 from *F. tricinctum*. Additionally, semipreparative liquid chromatography was used to purify ENs. MTT assays were used to conduct cytotoxicity tests. Cancer cell lines of human origin (epithelial colorectal adenocarcinoma cells, Caco-2) were only cytotoxicly affected by ENs A1 and B1 (Meca et al. 2010). The IC50 produced by EN A1 was 12.3 mM on Caco-2 cells, while the IC50 produced by EN B1 was 19.5 mM. Mycotoxins called enniatins have the potential to be cancer-fighting substances. The extracellular enzyme synthesis of amylase, lipase, pectinase, protease, cellulase, and laccase was examined in 50 endophytic fungi isolated from the medicinal herbs *Calophyllum inophyllum*, *Catharanthus roseus*, *A. calcarata*, and *Bixa orellana* (Sunitha et al. 2013). They stated that it was possible for these fungi to manufacture these enzymes. The numerous enzymes generated vary between different fungi and frequently depend on the host and other ecological elements. *Bacopa monnieri* is a medicinal herb that has been tested for its antibacterial properties against a variety of microbes by Katoch et al. It was researched how these endophytes interact ecologically with the host plant and their capacity to create the enzymes cellulase, protease, amylase, and lipase (Katoch et al. 2014). All endophytes displayed amylase activity. Notably, 98% demonstrated lipolytic, 28% cellulolytic, and 31% proteolytic activities. Devi et al. isolated the cellulase-producing *Penicillium* sp. endophytic fungus from the *Centella asiatica* plant (Devi et al. 2012).

12.3.2.2 Antifungal Properties of Secondary Metabolites

With cancer chemotherapy, allogeneic bone marrow transplantation, and organ transplantation, invasive fungal infections are significantly rising. Life-threatening fungal infections can be treated with a small selection of antifungal medications. Even though new antifungal medicines have been released on the market, the development of resistance to antifungal treatments is rising in patients receiving long-term treatment (Deshmukh and Verekar 2012). One of the most promising alternative sources for the separation of new metabolites for the treatment of fungal diseases is endophytic fungi. Endophytes from *Artemisia annua* were isolated by Liu et al., who then tested them against fungus known to damage crops, including *Gerlachia nivalis*, *Rhizoctonia cerealis*, *Helminthosporium sativum*, *Fusarium graminearum*, and *Gaeumannomyces graminis var. tritici* (Liu et al. 2001). The strongest antifungal activity was seen in ethanol acetate extracts. *A. indica*, *Holarrhena antidysenterica*, *Terminalia arjuna*, and *Terminalia chebula* are four

plants with significant therapeutic value that Tejesvi et al. identified as *Pestalotiopsis* strains. The maximum antifungal activity was demonstrated by their ethyl acetate extracts against six test species. A number of fungi were isolated and tested for their ability to inhibit a variety of pathogenic and saprophytic fungi (Tejesvi et al. 2007). These fungi produced six bioactive substances, including cerulenin, arundifungin, sphaeropsidin A, 5-(1,3-butadiene-1-yl)-3-(propene-1-yl)-2-(5 H)-furanone, and ascosterosides A and B. Arundifungin, 5-(1,3-butadiene-1-yl)-3-(propene-1-yl)-2-(5 H)-furanone, ascosteroside A, and ascosteroside B have antifungal properties on par with amphotericin, the standard treatment (Weber et al. 2007).

The medicinal plant *Michelia champaca* produces a variety of secondary metabolites with a wide range of pharmacological characteristics. This plant's endophytic fungus exhibits antifungal, anticancer, and acetylcholinesterase (AChE)-inhibiting properties. The activity of each extract was seen against two phytopathogenic fungi. Ethyl acetate extracts of the endophytic fungus *C. gloeosporioides* yielded one new compound, 2-phenylethyl 1H-indol-3-ylacetate, and seven known compounds, such as uracil, cyclo-(S*-Pro-S*-Tyr) (28), cyclo-(S*-Pro-S*-Val), 2-(2-aminophenyl) acetic acid, 2-(4-hydroxyphenyl)acetic acid, 4-hydroxy-benzamide, and 2-(2-hydroxyphenyl)acetic acid (Chapla et al. 2014).

Using NMR and X-ray crystallography, Li et al. isolated the endophytic fungus *Pestalotiopsis adusta* (L416) and discovered three metabolites that were derivatives of the chlorinated benzophenone, known as pestalachlorides A–C. *Fusarium culmorum* (CGMCC 3.4595), *Gibberella zeae* (CGMCC 3.2873), and *Verticillium albo-atrum* were three plant pathogenic fungi that the organic solvent extract from the fermentation broth significantly inhibited (CGMCC 3.4306). Their research also revealed that whereas pestalachloride B showed comparable action against *G. zeae*, pestalachloride A displayed strong antifungal activity against *F. culmorum*. *F. culmorum*, *G. zeae*, and *V. albo-atrum* were not resistant to the antifungal effects of pestalachloride C. Many endophytic fungi were isolated from the garlic plant by Shentu et al. (Ding et al. 2008). A significant antifungal efficacy against phytopathogens was demonstrated by these isolates. By using spectrum and mass data analyses, the bioactive metabolite generated by *Trichoderma brevicompactum* was identified as trichodermin as compared to the the compound's antifungal activity (Shentu et al. 2014).

12.3.3 Extraction of Metabolites from Endophytic Fungi

A vast range of secondary metabolites produced by filamentous fungi are known to be a rich source of biomolecules with potential medical uses. Secondary metabolites can be broadly categorized into four types based on their chemical structures and biosynthesis pathways: polyketides, non-ribosomal peptides, alkaloids, and terpenes. Plant endophytic fungi have the ability to produce a wide range of bioactive metabolites with different structural characteristics because of their unique living conditions. It has been shown that several of these metabolites have medicinal and

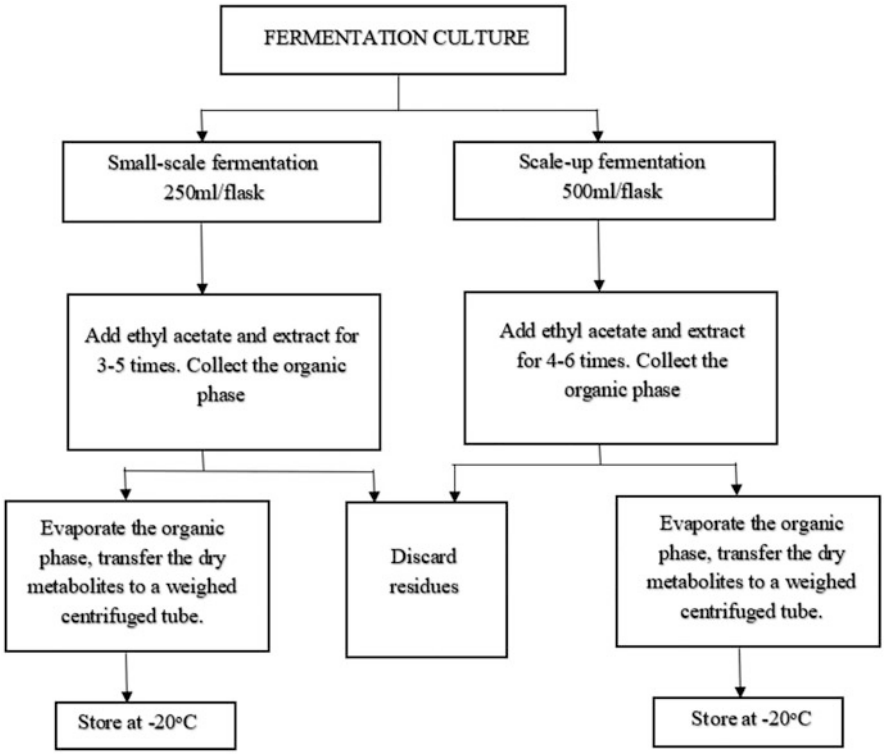

Fig. 12.2 Process of extraction of secondary metabolites from endophytic fungi

ecological relevance. Different techniques have been developed to isolate secondary metabolites from plant endophytic fungus utilizing high-performance liquid chromatography (HPLC) in order to find bioactive chemicals (Liu and Liu 2018) (Fig. 12.2).

The amount of spore pellet to be used determines how much 15% sterile glycerol (w/v) should be used; typically, a plate requires 100 L of glycerol. The fungus spores can survive for 2–3 years at −80 °C. A single colony is often obtained and used for subsequent tests after the fungal strains have been activated. Since different fungi have varying sporulation times, the incubation period for each can vary from day 5 to day 10. The majority of fungi typically undergo sporulation after a 7-day incubation period. The harvest time should be controlled in accordance with the production of desired metabolites since different fungi have varied growth rates and secondary metabolites may be produced either at the late stage of exponential growth phase or at the stationary growth phase. The majority of filamentous fungi are typically harvested after 7 days in order to extract secondary metabolites. However, in some circumstances, an incubation period of up to 20 days is used. The number of agar plugs that are incubated can be raised to 20–25 for fungi that develop slowly. In most cases, fungal growth enters the stationary growth phase after 48 h of liquid medium

fermentation. To make sure there is no contamination during fermentation, it is important to keep an eye on the fungal growth situation. A microscope can be used to observe contamination and determine its source. Typically, after 48 h of fermentation in liquid medium, fungal growth enters the stationary growth phase. For small-scale fermentation, the rice medium consists of 30 g of rice and 50 mL of double-distilled water. Following autoclaving for sterilization, the rice medium solidifies. After 30 days of fermentation, the rice medium and mycelia are mixed in solid-state fermentation. The solid-state cultures should be broken up into little bits for easier extraction. With a large pair of scissors or a pair of strong forceps, the cultures are minced in the fermentation flask. The colonies are given 100 mL of ethyl acetate for liquid-state fermentation. The amount of ethyl acetate needed for solid-state fermentation is around 100–150 mL; this ensures that the cultures are completely submerged. The ethyl acetate and culture mixture are put into a separatory funnel for liquid-state fermentation, where it is left to stand until the organic phase and aqueous phase are entirely separated. Pour out the organic phase for liquid-state fermentation once the lower phase has exited the separatory funnel. Since the mycelia and the rice remain in a solid state during solid-state fermentation, the organic phase can be drained off immediately. To prevent metabolite degradation, the water bath temperature for the rotary evaporator should be less than 40 °C. One of the fractions is that which was eluted using 30% EtOAc. This method can also be used to separate other fractions. We often use the maximum velocity to wash the column and collect the fractions because the flow rate is not constant. One drop is typically injected into the sample every 4–5 s. For additional analyses to determine the structure of the metabolites, the LC/MS and NMR are utilized. *Enterococcus* species, the second most common cause of hospital-acquired infections, are responsible for a wide range of illnesses, including infective endocarditis, bloodstream infections, and urinary tract infections. Vancomycin- and ampicillin-resistant bacteria include *Enterococcus faecium*. Additional fungi or bacteria are also an option. Depending on the goal of your research, choose several pathogenic bacteria or fungi. The initial concentration and subsequent dilutions of the metabolite might be downregulated depending on the solubility of the metabolite. The degree to which the color of the negative control changes determines the precise incubation period for bacteria and fungi. When the pink color vanishes is when it is best to act (Liu and Liu 2018).

A mycelial agar block taken from an active colony on a potato dextrose agar plate was used to inoculate 500 mL of potato dextrose broth with the endophyte for growth. The flask cultures were kept stationary for 2 weeks of incubation at 25 °C. Filtering the fungus broth culture allowed the cell-free supernatant to be extracted using ethyl acetate, chloroform, methanol, and hexane as solvents. An equivalent volume of solvent was added to the filter, and the mixture was violently agitated for 10 min. The sample was dried after the organic layer was separated using a separating funnel. For future usage, the dried residue was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL (Meenambiga and Rajagopal 2018).

12.4 Overview of Candidiasis

12.4.1 Virulence and Pathogenicity

Because of its virulence characteristics, candida actively contributes to the pathophysiology of infection's onset and progression. The initiation of an infection or colonization is caused by one set of virulence factors, while the spread of the infection is assisted by the other set (Deorukhkar and Roushani 2017). Due to changing circumstances, *Candida* spp. polymorphism suggests that it can transform from commensal to pathogenic form. It is distinguished by the morphological change from blastospores to hyphae, with pseudohyphae serving as the intermediary phase (Noble et al. 2017; Hanaoka and Domae 2021). One of the most important virulence factors of *C. albicans* is its capacity to develop true hyphae (San-Blas et al. 2000). Within the human microbiome, *C. albicans* can be found as yeast, and the change from yeast to hyphal form is a pathogenic transition (Mayer et al. 2013; Tsui et al. 2016). The hyphal form is invasive, and the cells penetrate the tissues of the host via active penetration which depends on the fungal activity and induced endocytosis that is achieved by hyphae invasion and is dependent on host activity. Hyphal development is influenced by a number of signaling pathways where the cyclic adenosine monophosphate protein kinase A (cAMP-dependent protein kinase A) is the most significant (Maza et al. 2017; Galocha et al. 2019; Lin and Chen 2018). A range of surface molecules of *C. albicans* interact with host ligands on the epithelial or endothelial surface that are crucial for mediating epithelial adhesion (Gale et al. 1998). Various factors may affect the adhesion process, such as types of proteins present in the cell wall, and cell surface's physiochemical properties. Adhesins on the yeast cell recognize ligands including sugar residues on human buccal epithelial cells and an array of extracellular matrix proteins such as fibrinogen, fibronectins, type I and type IV collagen, laminin, and the complement components like iC3b and C3d. Adhesins are the surface proteins that have a role in specific adhesion. A few well-studied adhesins of *Candida* spp. include those from the ALS (agglutinin-like sequence) family, Hwp (hyphal wall protein), EPA (epithelial adhesin) families, Int (integrin-like surface protein), and Mnt (-1-2-mannosyltransferase). The attachment of *C. albicans* to host epithelium is not entirely due to a single adhesin molecule, and other mechanisms of interaction with the host surface are hypothesized for this commensal organism (Deorukhkar and Roushani 2017; Hostetter 1994). It is a characteristic of *C. albicans* pathogenesis to produce biofilm. The majority of *C. albicans* infections result in severe morbidity and death due to the development of a biofilm on the host surface or on abiotic surfaces (implants) (Tsui et al. 2016). Although the biofilms produced by *C. albicans* are much denser and more complicated than those produced by other *Candida* spp., both *C. albicans* and *C. parapsilosis* attach effectively to the surface of catheters (Hawser and Douglas 1994; Ramage et al. 2005). Biofilm develops through several consecutive phases, beginning with the individual cells of *Candida albicans*, which form the basal layer. Cell proliferation and filamentation then occur, with hyphae

being the first step. In the maturation phase, an extracellular polysaccharide matrix is formed. Finally, the dispersion of non-adherent cells has the possibility of new biofilms and the dissemination in the tissue (McCall et al. 2019; Cavalheiro and Teixeira 2018). The extracellular matrix is composed of extracellular polymers and DNA involved in maintaining biofilm structure. DNA plays a vital role in binding the biofilm to the substrate, providing β-1,3-glucans which are essential for antifungal drug resistance. Biofilm channels facilitate cell supply with nutrients, air, and water, giving it new "multicellular" properties (Nett and Andes 2020; Talapko and Škrlec 2020; Li et al. 2020). Biofilm is caused by transcription factors such as Efg1, Bcr1, Tye7, cell wall proteins (Hwp1, Als3), and protein kinases (Cbk1, Ire1). These transcription factors are necessary for the expression of different genes for cell adhesion and filamentation in biofilms on abiotic surfaces. Adhesin Als3, the target of BCR1, plays a key role in the biofilm formation on abiotic surfaces (Ganguly and Mitchell 2011). Extracellular hydrolases are essential in the pathogenesis of candida, as they enable the invasion of host tissue by deranging the host cell membrane constituents especially in disseminated candidiasis. Phospholipases, secreted aspartyl proteinases, hemolysins, and lipases are mostly implicated in candida infections. The phospholipase enzyme in *Candida* spp. hydrolyzes the host cell membrane, revealing receptors to promote the adherence of yeast cells (Sardi et al. 2013; Ghannoum 2000).

12.4.2 Clinical Manifestations

The genus *Candida* has over 200 distinct species, but only a handful are opportunistic human pathogens which trigger infections once the host becomes emaciated or immunocompromised. Clinical manifestation of candidiasis may be superficial or invasive. Usually, superficial infections tend to affect the mucosal membranes and skin and can be effectively treated with topical antifungal medications. Invasive fungal infections, on the other hand, are frequently fatal, most likely as a result of inadequate diagnostic techniques and ineffective initial antifungal medications (Spampinato and Leonardi 2013). The most prevalent fungal infection of the oral cavity is candidiasis. Previous estimates suggest that 35–80% of the population carry oral candida. According to recent studies employing molecular detection techniques, the typical oral flora of all humans contains *Candida* spp. (Lewis and Williams 2017; Peters et al. 2017). *Candida albicans* is present in over 80% of oral fungal strains, making it the most common species in diseased and healthy mouths. *C. glabrata*, *C. parapsilosis*, *C. dubliniensis*, *C. tropicalis*, and *C. krusei* are also found in the mouth (Lewis and Williams 2017; Sav et al. 2020; Aslani et al. 2018). The highest incidence of diaper dermatitis in term newborns with 10% incidence and VLBW infants with 28% incidence occurs between the ages of 10–11 weeks and 7–9 months. Newborns who are diagnosed with diaper dermatitis showed intestinal colonization, with positive stool tests for a *Candida* species (Baley et al. 1986; Leyden 1986). Congenital candidiasis can be caused by a maternal infection or

massive exposure to maternal vaginal colonization during labor and delivery. Hematogenous dissemination, direct invasion of intact membranes, and ascending infection after ruptured membranes are key mechanisms for intrauterine infection. Although *C. albicans* is the species most frequently linked to congenital candidiasis, *C. glabrata* and *C. parapsilosis* have also been implicated with neonatal infections (Yadav and Prakash 2016; Whyte et al. 1982; Darmstadt et al. 2000). A distinct clinical condition called invasive fungal dermatitis has been identified in ELBW infants during the first 2 weeks of life. *Candida* species are commonly detected, but infection with other filamentous non-*Candida* fungal species, like *Aspergillus*, *Curvularia*, *Bipolaris*, and *Trichosporon*, produces analogous clinical array of lesions with erosive crusts (Rowen et al. 1995). In pulmonary candidiasis, although yeasts are frequently isolated from sputum or respiratory fluids, these isolates may not accurately reflect the pathogen that causes pulmonary infiltrates. It may be difficult to tell a deep-seated illness from a respiratory tract infection from positive cultures of specimens. Pulmonary candidiasis is either an impact produced by disseminated candidiasis or a localized infection brought on by aspiration, which is prevalent in patients (Meunier 1989). Esophageal candidiasis caused by *C. albicans* is the most prevalent kind of fungi-caused esophagitis. Patients with immunodeficiency and those with comorbidities are at increased risk of infection, which is either symptomless or manifested as acute odynophagia, dysphagia, and discomfort below the sternum (Mohamed et al. 2019; Rosołowski and Kierzkiewicz 2013). Yeast overgrowth within the gastrointestinal system is a primary cause of dissemination. In immunocompromised individuals, a novel clinical condition called focal hepatic candidiasis has been reported. Another kind of gastrointestinal candidiasis manifests as a fungus ball in the bile system, causing complete obstruction. Other rare types of candidiasis in patients with neoplastic disease are candidiasis peritonitis and intra-abdominal abscess (Meunier 1989). *Candida* species constitute a common cause of infant urinary tract infection, and renal candidiasis has been associated to it. Candidemia is frequently accompanied by renal parenchymal infiltration or fungus balls, which calls for systemic antifungal medication. Nonspecific symptoms of candida infection of the urinary tract in infants include fever, fatigue, apnea, abdominal distention, and significant gastric residuals. Acute renal failure is a typical clinical symptom of newborn renal candidiasis (Karlowicz 2003). *Candida* species invade the bones and joints as a result of hematogenous seeding or due to inoculation following trauma, intra-articular injections, a surgical operation, or injectable medication usage. Osteoarticular infections are frequently symptomatic months or even a year following a fungemia episode or a surgical operation (Johnson and Perfect 2007). *Candida* species are becoming more common reason for infections in neutropenic and non-neutropenic patients, increasing the risk of morbidity owing to invasive candidiasis and CNS involvement attributed to mycosis increase. Candida infections affecting the central nervous system are rare but occur most frequently in preterm newborns, who typically present with meningitis, micro- and macro-abscesses, and vascular and medullary damage (Henao and Vagner 2011). The growing frequency of invasive candidiasis in a variety of patient groups, including newborns, cancer patients, AIDS patients, and organ transplant

recipients, is a reason for worry in the medical community. The symptoms are varied, and distinct conditions such as localized and widespread infection must be evaluated independently. Similar disease manifestations may be caused by several yeast species.

12.5 Treatment for Candida Infections

12.5.1 Available Treatments

12.5.1.1 Therapeutic Application of Secondary Metabolites Against Candida Infection

Most *Candida* species are fluconazole-resistant and exhibit variable resistance to other antifungals, which has become a severe clinical challenge. Therefore, novel alternative antifungal strategies, including anti-candidal metabolites from endophytes and combination therapy, are required to improve patient outcomes due to the limited number of available treatment options and higher rates of therapeutic failures (Chowdhary et al. 2017). *Penicillium* sp., most likely *P. brevicompactum*, is one among the endophytic fungi identified within the inner bark of a yew tree in the Northwest Pacific that produced bioactive metabolites. The fungus was cultured and extracted with methylene chloride, to yield the compounds mycophenolic acid and ergosterol peroxide which showed activity against *C. Albicans* (Stierle and Stierle 2000). A study in 2007 isolated the fungi *Ascomycota* and *Basidiomycota* from their fruit bodies or from soil. In all, 1510 different fungus strains were cultivated in submerged culture, and then the extracts of the mycelium and culture filtrate were evaluated for resistance to *Candida albicans*. Five endophytic strains were chosen for the isolation of active principles due to the contribution endophytes provide to the overall fungal biodiversity and their bioactive compound production. The endophyte *Phomopsis* sp. produced a compound cerulenin which is a fatty acid and polyketide synthase inhibitor that showed anti-candidal activity. Another isolate identified as *Gnomoniaceae* produced ascosteroside A, which exhibited anti-candidal activity that is known to be attributed to the inhibition of b-(1,3)-glucan synthesis. Ascosteroside B analogous to the former was also obtained. Arundifungin, generated by the isolate *Amphisphaeriaceae* in *Xylariales* order, is structurally and functionally linked to ascosterosides which serve as b-(1,3)-glucan synthesis inhibitor. An endophyte isolated from an asymptomatic *Quercus ilex* leaf varied at only one position from *Gnomoniaceae* that produced sphaeropsidin A. Sphaeropsidin compounds are pimarane diterpenes that are phytotoxic and fungitoxic and have been implicated in the development of symptoms and the repulsion of competing phytopathogenic fungi, like cerulenin. The strain, which was isolated as an asymptomatic endophyte from *Cistus salviifolius*, belonged to the *Sarcosomataceae* (order *Pezizales*). Due to its anti-candidal efficacy, it was isolated and had similar activity identical as sphaeropsidin A (Weber et al. 2007).

For the first time, the metabolites of the endophytic fungus *Penicillium* sp. from *Hopea hainanensis* leaves have been described. Through bioassay-guided fractionation, the EtOAc extract of a solid-matrix stable culture from this fungus generated six metabolites, which were identified through a combination of spectral and chemical analysis to be monomethylsulochrin, rhizoctonic acid, asperfumoid, physcion, 7,8-dimethyl-isoalloxazine, and 3,5-dichloro-*p*-anisic acid. All six compounds were tested for in vitro bioactivity, including their ability to inhibit three human pathogens including *Candida albicans* species. The growth of *C. albicans* was suppressed by compounds physcion, rhizoctonic acid, asperfumoid, and 3,5-dichloro-*p*-anisic acid with minimal inhibitory concentrations (MICs) of 50.0, 40.0, 20.0, and 15.0 g/mL, respectively (Wang et al. 2008).

A cytochalasan derivative called phomopsichalasin has a unique ring structure with an isoindolone moiety attached to a 13-membered tricyclic framework. It was discovered in an endophytic *Phomopsis* species that was found on *Salix gracilistyla*, a willow plant. Cytochalasans are well-known actin-binding fungus metabolites. Phomopsichalasin has been demonstrated to have inhibitory properties towards the human pathogenic fungus *Candida tropicalis* and other bacterial pathogens. Another compound 1α-10α-Epoxy-7α-hydroxyeremophil-11-en-12,8-β-olide was found from *Xylaria* sp. BCC 21097, which was isolated from palm *Licuala spinosa*. It shares structural similarities with eremophilanolide sesquiterpenes. *Candida albicans* was successfully eradicated by the substance. The fungi *C. albicans* and *Candida utilis*, as well as the bacteria *S. aureus*, *B. subtilis*, *M. luteus*, *E. coli*, and *Pseudomonas aeruginosa*, were shown to be inhibited by the compound altersolanol A (Mousa and Raizada 2013). A study isolated two novel cytotoxic and antifungal compounds from the metabolites of the endophytic fungi *Dendrobium officinale*, (4S,6S)-6-[(1S,2R)-1, 2-dihydroxybutyl]-4-hydroxy-4-methoxytetrahydro-2H-pyran-2-one and (6S,2E)-6-hydroxy-3-methoxy-5-oxodec-2-enoic acid, and three other known compounds, LL-P880γ, LL-P880α, and ergosta-5,7,22-trien-3b-ol. Spectroscopic techniques were used to identify the chemical structures. Cytotoxicity and antifungal effects were assessed for all isolated compounds 1–5. All the pathogens evaluated, including *C. albicans*, exhibited considerable antifungal activity for compounds 1–4 with MIC of 50 g/mL) (Wu et al. 2015).

12.6 Conclusion

Endophytic fungi generate a wide range of secondary metabolites with intriguing applications in several industries. It has been demonstrated that a number of these metabolites exert antibacterial, anticancer, antifungal, and antioxidant properties. They have also been employed in the manufacture of pharmaceuticals and bioactive substances for the agricultural and pharmaceutical sectors. Another fascinating and important study field is the exploitation of these metabolites to control and to treat many fungal diseases including candidiasis. Candidiasis is a public health issue with high morbidity and mortality rates due to the growing frequency of invasive

candidiasis in a variety of immunocompromised patient groups, including newborns, cancer patients, AIDS patients, and organ transplant recipients. A range of metabolites that act against candida have been identified in the past few years, and currently a lot of novel compounds from endophytic fungi are screened both in silico and in vitro. As a result, it is anticipated that future research on endophytic fungi and their secondary metabolites would increase, leading to the identification of new bioactive substances and their uses.

12.7 Future Perspectives

One of the key sources for obtaining new bioactive substances is endophytic fungus. Endophytic fungi in their bioactive form have anti-candidal activity that is controlled by several pathophysiological processes associated with the development of candidiasis. Further research and explanation are required in studies relating to the safety concerns for the long-term use of endophytic fungi's bioactives and their interactions with other medications. Future study in this field is therefore required to confirm the efficacy of these medicinal substances derived from endophytic fungus and their products as prospective medications or nutraceuticals for the treatment of candidiasis. More research is needed to learn more about this underutilized resource for the creation and isolation of new molecules against candidiasis with therapeutic relevance and biochemical and pharmacological potential. The bioactive metabolites produced by endophytic fungus have been widely studied and published in recent years. These substances can be exploited to create brand-new natural products with therapeutic uses. Nearly 270,000 vascular plant species serve as the host for more than 1.5×10^6 endophytic fungi; therefore, new discoveries of their metabolites would be a viable course of action (Agrawal et al. 2022).

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