Chapter 2

Natural Products of Fungal Endophytes and their Therapeutic Potential: A Focus on Cardiovascular Disease

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Abstract

Fungal endophytes are the microorganisms that live asymptptomatically within the plant tissue without causing any apparent damage to the plant body. These endophytes are the epitomes of numerous natural products and secondary metabolites, which have an application in the medicinal, agricultural, or industrial arena. Since the advent of taxol production from endophytic fungi Taxus brevifolia, fungal endophytes have generated significant interest in the healthcare industry. From antibiotics to antiviral compounds; antioxidants to anticancer agents, the secreted products of fungal endophytes have emerged as a potential substitute for the conventional medicine. It is noteworthy that, the changing lifestyles have shown an upsurge in ailments like cancer, asthma, chronic obstructive pulmonary disease, and cardiovascular diseases (CVD). Although these diseases have been curbed to a large extent, but CVD is still a global burden which alone claims 17.5 million lives every year. The conventional synthetic drug therapy has proven vague, hence, there is a general call to identify new cardioprotective agents from microorganisms that are highly effective with minimal side effects. It is estimated that there are nearly 20,00,000 natural products of microbial origin, of which nearly 23% have cardio-protective properties. Hence, the intent of this review is to provide an insight on novel aspects of some natural products produced by fungal endophytes in nature that may have significant therapeutic potential in CVD management and hence, facilitate the product discovery processes.
Introduction

The burden of cardiovascular disease (CVD) is ever growing and it is estimated that 17.5 million people die every year due to this disease. Due to the diverse risk factors (high LDL-cholesterol, diabetes, hypertension, physical inactivity, smoking, obesity or use of alcohol), the CVD is the leading cause of mortality in the United States for both men and women [1]. Though with the use of conventional medicines the death rate has declined to a significant level, but the burden of this disease still remains high. As conventional therapy evolved a placid response, a systematic search for an alternative form of medicine or new drug entity with an aim to manage the CVD in a better way, is a need of an hour. In a plethora of alternative medicines, secondary metabolites or natural products are tried as anticancer drugs, antiviral agents and few have emerged as a worthy immunomodulators [2,3]. However, the use of fungal natural products as therapeutic agents in CVD management is still a matter of discussion.

Fungal endophytes are the major producers of natural products besides plants and marine macro-organisms. The term “fungal endophytes” includes a group of fungi that grow and colonize the internal plant tissues beneath the epidermal cell without causing any apparent symptoms or damage to the host plant and are recognized as the rich sources of bioactive natural products [4]. The mutual interaction between endophytes and the host plant results in fitness benefits for both partners and may provide a defense and survival conditions to the host plant by generating a plethora of metabolites which, when isolated and characterized, may serve as a potential substance for use in medicine, agriculture, and industry. The making of bioactive compounds by endophytes is directly associated with the independent evolution of these microorganisms within the host plant, which may have assimilated the genetic information from the host tissues through the horizontal gene transfer, permitting them to better acclimatize within the host plant. Fungal endophytes produce varied bioactive natural products including steroids, terpenoids, alkaloids, tetralones, benzopyranones, phenolic acids, besides xanthones, chinones, quinones, flavonoids, and others, which are promising in human health concerns [3]. Such bioactive metabolites find a wide-ranging application like antibiotics, immunosuppressants, antiparasitics, antioxidants, and anticancer agents, though a few of them like statins, have been used as golden standards in cardiovascular research and have evolved as an alternative therapeutic agent for CVD management. Statins are wonder drugs that have redesigned the treatment of hypercholesterolemia and allied cardiovascular diseases. These wonder drugs are produced as natural products by certain endophytes like simvastatin by Aspergillus terreus; mevasstatin by Penicillium citrinum; and lovastatin by Pleurotus ostreatus. Although statin production from endophytes is in dearth, they have been successfully used as derivatives for the synthesis of other statins. The intent of this chapter...
is to deliver insights into the presence of various natural products produced by the endophytes in nature and how some of these natural products could be used for targeted therapeutics in CVD management.

**Fungal Endophytes as Producers of Natural Products**

The advent of fungal endophytes came into attention only when Freeman in 1904 presented pieces of evidence about the presence of fungus in an annual grass. Later scientists around the world started to explore the folklore of this new entity from almost all plant populaces in nature from different plant parts, geographical niches and diverse environmental conditions and sites [6–8]. Subsequent studies have shown that the endophytic association with the host plant influences the ecology and evolution of both fungal endophytes and their host plant which empowered them to create the wide host range. Fungal endophytes are not host specific rather a single endophyte may colonize different host plants or even can be isolated from different plant tissues of the same plant [9] [Figure 1].

Reported estimates say that there are more than 20,000 bioactive metabolites which are of microbial origin [10]. Among all producers, fungal endophytes are the most important recognized eukaryotes that are well-known producers of numerous putative and novel metabolites which are straightway used as drugs or function as lead structures for synthetic modifications [11–13]. According to the estimates of Hawksworth and Rossman (1987), there may be as many as 1 million different fungal species, yet only about 100,000 have been defined. Though evidence of endophytes being recognized as major producers of metabolites accumulates day by day, yet only 8.9% of defined endophytes are explored in the natural product industry [14]. Thus, it seems that the discovered percentage of economically viable fungal metabolites is scantly and further needs a good technological approach.

**Figure 1:** An assortment of endophytic fungi recovered from the foliage of *Asparagus racemosus*.

The successful discovery of numerous drugs from microbial origin like antibiotic penicillin from *Penicillium* sp., the immunosuppressant cyclosporine from *Cylindro-
carpon lucidum, the antifungal agent griseofulvin from *Penicillium griseofulvum*, micafungin, an anti-fungal metabolite from *Coleophoma empetri*, mycophenolate used as immunosuppressant in renal transplant produced by *Penicillium brevicompactum*, illudin-S, an anti-cancer sesquiterpenoid from *Omphalotus illudens*, β-lactam antibiotics from various fungal taxa, and/or cefditoren pivoxil, a broad-spectrum antibiotic derived from *Cephalosporium* sp., has shifted the focus of drug discovery from plants to microorganisms [15–18]. Most of the microbial origin drugs established are either anticancer, immunosuppressant, antifungal, antiviral, or antioxidants but very few have been established in CVD treatment. For example, the cholesterol biosynthesis inhibitor lovastatin from *Aspergillus terreus*, rosuvastatin from *Penicillium brevicompactum* and *Penicillium citrinum*, used for treating dyslipidemias [19] and paracin from *Cephalosporium* sp., used as a cyclooxygenase-2 inhibitor in atherosclerosis-mediated inflammation are just a token of endophytic origin drug leads which are used to treat CVD. The described populations of endophytic strains with respect to CVD treatment are few, however, the opportunity to find new strains and targeting their natural products of cardioprotective interest, will pave new avenues and milestones in treating various ailments of CVD.

**Natural Products as Alternative Medicines**

Ancient people, in past times, realized that different plant parts like root, leaf, or stem concoctions had an impending effect in curing some diseases. These plant concoctions improved the quality of life, acted like an anesthetic and reduced the pain, and provided long-term relief in various ailments. Although these plant mixtures had a propounding effect on the human health, the chemical nature of bioactive compounds in these complex mixtures and how they functioned, was elucidated much later. It was not until Pasteur postulated that fermentation process is caused by some living entities that people seriously initiated to investigate these living entities as a source for bioactive natural products. Then, observational serendipity and the command of opinion provided the impetus to Fleming to usher in the antibiotic era via the discovery of penicillin from *Penicillium notatum*. Since then, people have been engaged in the discovery and application of microbial natural products with activity against both plant and human pathogens.

Natural products are chemical compounds or by-products derived from microorganisms, plants, or animals [5]. Endophytes provide a broad variety of bioactive secondary metabolites with a unique structure, such bioactive metabolites find wide-ranging application such as antibiotics, immunosuppressants, antioxidants, antipara-
sitics, and anticancer agents besides acting as cardioprotective agents [11]. An exemplary progress has been made in product chemistry for the last few decades, which lead to an impression on contemporary medicine since about 40% of prescribed drugs are based on natural products. Additionally, 49% of the new chemical products registered by the Food and Drug Administration (FDA) are natural products or derivatives thereof [20]. As per the recent reports, between 1989 and 1995, 60% of approved drugs from FDA and pre-new drug candidates were natural derivatives [21], and from 1983 to 1994, over 60% of drugs approved as cancer drugs or cancer drugs candidates were of natural origin [22]. In fact, the world’s first billion-dollar drug, paclitaxel (Taxol) an anticancer drug, is a natural product derived from the yew tree and few fungal endophytes from the group Taxomyces [23]. Literature regarding the use of natural products from fungal endophytes as cardioprotective agents is scanty and only a few have been explored in cardiovascular research till date. Products like lovastatin and rosuvastatin are lipid-lowering drugs isolated from fungal endophytes [19]. Likewise, phenolics and flavonoids of Xylaria sp., Colletotrichum sp., and Pestalotiopsis microspora exhibit a strong cholesterol lowering effect. Besides the hypolipidemic effect, these products have strong antioxidant effects and prevent further reactive oxygen species (ROS) generated cellular damage within the intima of the vascular wall [24]. Most of the cardioprotective natural products are bestowed with antioxidative properties. In one of our study, we have shown that the aqueous extract of Terminalia arjuna displays anti-atherogenic effects in an atherogenic rabbit model system [25]. Whether, metabolites produced by the endophytes within the tissues of Terminalia arjuna, do exert a role in anti-atherogenesis, needs to be evaluated. In the next section, are highlighted and illustrated few endophytic origin natural products which are being used as cardioprotective agents in the modern civilization.

Lovastatin

Lovastatin (C_{27}H_{36}O_{5}), [Figure 2] is a polyketide-derived natural product isolated from Aspergillus terreus and Pleurotus ostreatus with a commanding inhibitory effect on 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase [26]. Lovastatin production is also reported in Aspergillus niger, Aspergillus flavus, Penicillium sp., Trichoderma viride, Monascus ruber, and Monascus sp., endophytes [27]. Immediately after its discovery in 1970, this metabolite was taken into clinical development as an impending drug for lowering LDL cholesterol. After its successful clinical trial, lovastatin received the approval in the USA in 1987 and is believed to be the first statin approved by the FDA, which comes under various brand names like Mevacor, Altocor, Altoprev, and Advicor [28–29]. Experimental evidence from literature reveals that at least 18 proteins are involved in the lovastatin biosynthesis process and acetate is the major initiator molecule in the production process [30].
Figure 2: The structures of some compounds isolated from endophytic fungi which are used in CVD treatment and management. A) Lovastatin; B) Pestacin; C) Simvastatin; D) Ternatin; E) Nicotinamide riboside; F) Mevastatin; G) Isopestacin and H) Graphislactone A.

Lovastatin is a pro-hypolipidemic metabolite that inhibits HMG-CoA reductase, a key enzyme that catalyzes the conversion of HMG-CoA to mevalonate in the cholesterol biosynthesis pathway. Mevalonate is an obligate precursor required for cholesterol biosynthesis and lovastatin impedes with its production by binding to the HMG-CoA reductase as a reversible competitive inhibitor of HMG-CoA. Lovastatin in its native state is an inactive lactone-form pro-drug, once hydrolyzed, forms an active form. The in vivo hydrolysis of the gamma-lactone closed ring-form releases the β-hydroxy acid open ring form, which is the active form of this drug [31]. Though lovastatin is a well-tolerated drug in the body from the physiological point of view, it still incurs few side effects and contraindications. For example, its continued use elevates creatine phosphokinase levels, causes flatulence, indigestion, constipation and muscle pain. Unlike other statins it rarely causes dermatomyositis, hepatotoxicity or severe myopathy. Lovastatin is contraindicated during pregnancy as it may cause birth defects such as bone abnormalities or learning disabilities due its potential to disrupt the lipid metabolism. Lovastatin has been studied for their chemotherapeutic effects in certain types of cancers. The clinical data from these studies could not demonstrate any encouraging results, however, these studies have shown that lovastatin reduces the proteasome activity within the active dividing breast cancer cell lines, independent of their HMG-CoA reductase inhibition [32].

Compactin

Mevastatin (ML-236B) or Compactin (C_{23}\text{H}_{34}\text{O}_{5}) [Figure 2] is a cholesterol-lowering agent isolated from Penicillium citinum, Pythium ultimum, Penicillium cyclopium, and a few strains of Colletotrichum sp., [30]. Apart from its hypolipidemic action, mevastatin reduces stroke damage and augments endothelial nitric oxide synthase, while few studies found it a vital antioxidant in their studies [33]. Akira Endo and his associates carried an in vitro studies on more than thousand different strains of endo-
phytes for their ability to produce an inhibitor of sterol synthesis. The investigating team discovered three active compounds, which were later designated as ML-236C, ML-236A, and ML-236B in the culture broth of the fungus *Penicillium citrinum*. The main compound ML-236B, also called as mevstatin, is identical to another compound which was isolated later from *Penicillium brevicompactum* by an individual British group [34]. ML-236B is a specific inhibitor of HMG-CoA reductase and highly effective in lowering plasma cholesterol levels.

Mevastatin competitively inhibits HMG-CoA reductase with a binding affinity, counted 10,000 times greater than the HMG-CoA substrate. Mevastatin, like other naturally occurring statins, is an inactive prodrug that is activated by *in vivo* hydrolysis of the lactone ring [35]. The hydrolyzed lactone ring imitates the tetrahedral intermediate produced by the reductase allowing the agent to bind with greater affinity than its natural substrate. The bicyclic portion of the mevastatin binds to the coenzyme A portion of the active site and hence imparts its function. Mevastatin has served as one of the lead metabolites for the development of the synthetic compounds like pravastatin, and even few fungal strains like *Penicillium citrinum* and *Penicillium cyclopium* have been genetically mutated to enhance the mevastatin production in fermentation batch cultures [36].

It is presumed that mevastatin is considerably safe when compared to other types of statins because of its simple structure and hence causes least contraindications and adverse events. Accept myopathy, no other worrying side effects were reported in study subjects with the continuous use of this drug.

**Simvastatin**

Simvastatin \((\text{C}_{25}\text{H}_{38}\text{O}_{5})\) [Figure 2], sold under the trade name Zocor, is a lipid-lowering cardiovascular drug produced by an endophytic fungus *Aspergillus terreus*, as a fermentation product [37]. Alike other statins of endophytic origin, simvastatin inhibit hepatic HMG-CoA reductase in a competitive fashion and reduces the serum lipids to a larger extent. Evidence from the literature suggests that its intake along with exercise and low lipid diet, display propounding results in lowering the elevated cholesterol levels. Apart from acting as hypolipidemic agent, this product also acts as an immunomodulatory agent and modulates immune response via suppression of major histocompatibility complex II on antigen-presenting cells like human vascular endothelial cells [38].

The main use of simvastatin is to treat dyslipidemia and to prevent atherosclerosis-related complications like stroke, myocardial infarction or heart attacks in individuals who are at high risk. However, few studies suggest that it is also used as a trial candidate in the management of cancer [39]. In the 4S (Scandinavian Simvastatin Survival Study) study, a randomized clinical trial carried for 5 years, simvastatin reduced overall mortality in individuals
with existing CVD and high LDL-cholesterol by 30% and cardiovascular mortality by 42%. The study also shows that the risks of heart attack, stroke, or requiring a coronary revascularization procedure were reduced by 37%, 28%, and 37% respectively [40]. In the Heart Protection Study carried for 4.5 years on a diverse set of subjects including the women, the elderly, and patients with diabetes, evaluated the effects of simvastatin in people with risk factors including existing CVD, diabetes, or stroke but having relatively low LDL cholesterol. This trial showed that simvastatin reduced the overall mortality by 13% and cardiovascular mortality by 18% [41]. The same study also demonstrated that people receiving simvastatin experienced 38% fewer non-fatal heart attack.

Simvastatin is contraindicated with pregnancy causing severe birth effects like lung immaturity and mental retardation, whereas it is highly recommended that simvastatin uptake should be avoided during breastfeeding, as it severely disrupts the lipid metabolism of the baby [42]. Other side effects may include joint pain, muscle cramps, indigestion, and eczema.

**Ternatin**

Natural products are known to influence several cellular processes in the body by modulating protein targets. Besides *Clitoria ternatea* plant, various fungal endophytes like *Aspergillus* sp., *Penicillium* sp., *Chaetomium* sp., accumulate a group of polyacylated anthocyanins, named ternatins [Figure 2]. These compounds suppress hyperglycemia, hence exhibit its antidiabetic activity via increased insulin release and enhanced antioxidant defense. This way ternatins reduce the influence of the one of the risk factor of CVD [43].

Apart from hypoglycemic properties, ternatin polyphenols have exhibited an anti-inflammatory property in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells, which was demonstrated by Nair et al (2015) [44]. A strong inhibition of COX-2 activity and partial inhibition of reactive oxygen species (ROS) was demonstrated by a flavonol fraction of ternatin. The anthocyanin fraction was shown to inhibit iNOS protein expression, and nitric oxide production independent of ROS inhibition and also suppressed nuclear NF-κB translocation. The authors suggested their use as drugs or nutraceuticals for protection against chronic inflammatory diseases by suppressing the excessive production of pro-inflammatory mediators from macrophage cells [44]. This study provides a possible hint that ternatins could act as a potent mediator in the macrophage-laden inflammasome milieu.

Ternatin is shown to possess cytotoxic and anti-adipogenic properties. Shimokawa et al (2007) demonstrated that a highly methylated cyclic heptapeptide (-)-Ternatin (1), significantly inhibited fat accumulation in 3T3-L1 cells (EC50=0.14 microg/ml). In high-fat-fed mice, treatment with 1 at 5 mg/kg/day significantly suppressed an increase in body weight and fat accumulation [45]. Ito et
al examined the mechanism used by (-)-ternatin and [l-Ala(4)]ternatin, an inactive analog of (-)-ternatin to inhibit adipocyte differentiation. The authors determined the expression of adipocyte markers and lipogenic enzymes and found that (-)-ternatin significantly attenuated the mRNA expression of several adipocyte markers in a dose-dependent manner, while [l-Ala(4)]ternatin showed no effects. The authors suggested that (-)-ternatin showed its effects on mid-to-late differentiation stages of adipocytes. Also, (-)-ternatin potently inhibited triglyceride synthesis in adipocytes and primary hepatocytes [46]. The role of ternatin has also been studied in ischemia/reperfusion injury. Guimaraes, et al in their study induced ischemia/reperfusion injury in rat testis and demonstrated that bioflavonoid ternatin (TTN) pretreatment before I/R injury showed antiperoxidative and antioxidative properties [47].

From the above-mentioned evidence available from in vitro and in vivo studies in literature, it is clear that ternatin possesses potent anti-inflammatory, antioxidative and antiadipogenic properties besides inhibiting migration and proliferation of cells. Based on these properties, researchers have advocated its use in stress-related and metabolic disorders. So far its role in cardiovascular diseases has not been explored. Since inflammation and oxidative stress, as well as obesity, are the key factors implicated in the pathophysiology of CVD, it is natural that studies should be conducted with this natural cyclic peptide to exploit its virtues in experimental and clinical studies and find its therapeutic targets.

Nicotinamide Riboside

Nicotinamide, nicotinic acid, nicotinamide riboside (NR) [Figure 2] and nicotinic acid riboside (NAR) are the major precursors for nicotinamide adenine dinucleotide (NAD) biosynthesis in humans. NAD biosynthesis is a highly regulated process, responsive to many biological cues and NAD is essential for cellular homeostasis and serves as a sensor of the metabolic state of the cell. This way NR impacts several biological pathways eg., cell survival, energy metabolism, aging and cell signaling [48]. NR is isolated from few endophytes of Bacopa monnieri and Azadirachta indica plants. Besides Saccharomyces cerevisiae, the most prominent among NR producers are Priformospora sp., Colletotrichum sp., and Epichole sp., [49].

Although, NR does not play a solo role in cell regulation or metabolism, it exerts its beneficial effects via NAD. The latter is mediated by the enzymes like poly(ADP-ribose) polymerases, mono-ADP-ribosyltransferases, and the sirtuins. All these enzymes are involved in protein modifications, stress resistance, metabolism, and endocrine signaling, suggesting that these enzymes and/or NAD(+) metabolism could be targeted for the therapeutic benefit of CVD. However, till date there is no practical advance on this hypothesis, whether the enzymatic pathway, mediated by NR or its enzymes involved in the pathogenesis of CVD, could be utilized as a therapeutic ap-
approach towards CVD management. Despite scarce leads, few studies do suggest that NR is involved in promoting insulin sensitivity, mitochondrial biogenesis, and sirtuin functions. It is shown to negate the effects of high-fat diet and is also shown to be neuroprotective [50]. Low-grade chronic inflammation (metaflammation) is a characteristic feature of metabolic diseases, such as type 2 diabetes, obesity, cancer and cardiovascular disease. Lee et al. (2015) demonstrated that NR in a dose of 100 mg/kg/day given for 7 days to a rat model of type 2 diabetes attenuated hepatic metaflammation. Further, NR treatment was shown to significantly improve hepatic proinflammatory markers, including tumor necrosis factor-alpha, interleukin (IL)-6, and IL-1 [51]. In a mice model of cardiomyopathy lacking transferrin receptor Tfr1 in the heart, administration of nicotinamide riboside was shown to prevent against cardiomegaly, poor cardiac function, mitochondrial respiration, and impaired mitophagy.

As outlined above, the evidence in the literature clearly suggests that NR being a natural precursor of NAD+ has the powers of being a very effective nutrient and can provide protection against several metabolic and age-related disorders eg. cardiovascular disease, obesity, diabetes, cancer and neurodegenerative that are influenced by high-fat diet, inflammation, mitochondrial dysfunction, etc. and thus NR shows the promise to be used as a treatment strategy towards the protection of CVD.

Antioxidant Compounds

Fungal endophytes are considered as the finest antioxidant producers after herbs. This potential is attributed to their ability to produce phenolics, terpenoids, and flavonoid compounds, which have the scavenging effects on free radicals and reactive oxygen species. The significance of natural products possessing antioxidant activity basically lays in the fact that they are exceptionally effective against the damage caused by ROS and oxygen-derived free radicals. The generation of the free radicals and ROS within the different cells of the heart contributes to various pathophysologies of CVD, for instance, atherosclerosis, ischemia/reperfusion injury, hypertension, or diabetes mellitus. The antioxidant compounds sequester the generated reactive ROS and free radicals, which otherwise lead to the oxidative modification of lipoproteins that may lead to damage the cellular structure. These antioxidant compounds besides acting as cardioprotective agents, also possess anti-inflammatory, antitumor, antiviral, an antibacterial activities [52].

In a study, Liu et al (2007), isolated *Xylaria* sp., from the medicinal plant *Ginkgo biloba*, that exhibited a strong antioxidant activity. The methanolic extract of this endophyte exhibited a strong antioxidant potential because of the presence of phenolics and flavonoids compounds [53]. Pestacin and isopestacin are the two important antioxidants which are produced by the endophytic fungus *Pestalotiopsis microspore*, isolated from the plant, *Termi*
nalia morobensis [Figure 2]. Due to their structural similarity with the flavonoids, both the compounds scavenge superoxide and hydroxyl free radicals in the presence of water molecules. Besides their antioxidant activity, these two compounds also shown to exhibit antimycotic and antifungal properties [54]. In a similar fashion Graphis lactone A, a phenolic metabolite [Figure 2] isolated from the endophytic fungus Cephalosporium sp., was shown to exhibit a strong antioxidant and free radical-scavenging activities in vitro more efficiently than the celebrated effects of ascorbic acid and butylated hydroxytoluene [55].

**Natural Product Collection and Isolation Techniques**

Plants bearing ethnomedicinal properties, possess higher endophytic load than those plants which own less or no medicinal properties, the rationale of which is still a matter of deliberation. Endophytes can be isolated from any plant part, like leaf, stem or root, though the former is the most preferred plant part, due to the rich endophytic diversity within its tissues.

Once the plant sample (part of a root, leaf or stem) is selected, it is cut into small pieces and placed in a sterilized sealed plastic bag, excess moisture is removed and stored at 4°C, if not proceeded on the same day. The plant material is thoroughly surface sterilized with 70% ethanol, to remove the epiphytes and finally they are air dried under a laminar-flow hood. Then, with a sterile blade, tissues are cut into small pieces with 1–3 mm³ dimensions and placed onto a potato dextrose agar (PDA) medium amended with chloramphenicol to inhibit the bacterial growth. After several days of incubation, hyphal tips of the fungi are removed and transferred to the PDA, identified via morphological and molecular biology techniques and finally grown in pure cultures. Eventually, the identified endophyte is tested for its ability to produce secondary metabolites or natural product, by growing in a shake or still culture with varying media and growth conditions. Ultimately, once appropriate growth conditions are found, the endophyte is fermented, extracted and the bioactive compound(s) is isolated by polar/non-polar or neutral solvents in different propositions and finally characterized by various spectroscopic techniques. It is important to mention that all sorts of common or advanced procedures are explored for the product isolation, identification, and characterization, in order to obtain the product(s) of importance. Needless to say, at this point, one cannot put an emphasis on the isolated compound unless and until it is not tested for any bioassay method. Because, central to the isolation process, bioassays guide the compound purification processes and further help in establishing a translational approach. Further, bioassays are important in decoding and depicting the natural-product activity within the target system. The target system can involve organisms, animal models, tissues, enzymes or their analogs, or model chemical systems that relate to the purpose for
which the new compound is needed [56]. The workflow depicting the discovery of a natural product from endophytic fungi is depicted in Figure 3.

Figure 3: The practical workflow depicting the framework for the discovery and identification of a natural product/secondary metabolite from endophytic fungi.

**Conclusion**

Fungal endophytes are the least studied and investigated group than the other microorganisms of same ecological niche. They act as a rich and reliable source of novel bioactive compounds with a prospective for exploitation in medical, agricultural, or industrial arenas. It is imperative to understand the ways through which endophytes exist and affiliate to their surroundings, in order to be more accurate and extrapolative about which host plants to search for the study and spend time isolating endophytic microflora components. This may expedite the natural product discovery processes.

Although, work on the utilization of natural products in the medicinal arena has begun, but their exploration towards the CVD management is still lukewarm. Natural products of fungal endophytes are widely used as antibiotics, antiviral, anticancer, antimicrobial, and antifungal agents, but barring few statins like lovastatin, mevastatin, and simvastatin, fungal metabolites are the least studied compounds in cardiovascular research. However, the naturally occurring statins have proved as a reliable standard for the synthesis of other synthetic statins like atorvastatin, cerivastatin, or fluvastatin. In this context, the use of natural products of endophytes as mediators in the process of CVD treatment and management assumes greater importance. Nevertheless, much of the natural products exhibit an antioxidant activity, but only a few have yet been tested for their possible role in CVD prevention and
treatment. As the studies on this huge poorly exploited resource of microorganisms have just begun, it has already become evident that a huge potential for the organism, its products, and an impending functional discovery towards CVD management holds a great promise.

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