CHAPTER- II

REVIEW OF LITERATURE

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2.1 Endophytes

The term "endophyte" has been derived from two Greek words "endon" meaning within or inside and "phyton" meaning plant. Endophytes therefore represent the type of microorganisms that inhabit intercellular and intracellular healthy issues of plants during any part or throughout their complete lifecycle. They do not show any apparent disease symptoms or negative effects on the host plants they dwell within. De Bary (1866) provided the first definition of an endophyte, as "*any organism that grows within plant tissues are termed as endophytes*". However, the definition continues to change as per various researchers (Wilson, 1995; Hallmann *et al*., 1997; Bacon and White, 2000). The most suitable definition for endophytes has been provided by Petrini (1991) which states that any organism that at some part of its life cycle colonizes the internal plant tissues without causing any type of harm to the host plant is an endophyte. They have been also defined as a group of microorganisms, mostly bacteria or fungi, which colonize the intercellular and intracellular parts of plants (Pimentel *et al*., 2011; Singh and Dubey, 2015). These organisms complete all or parts of their life cycle within the host plants without causing any apparent disease symptoms. They are ubiquitous in nature and exhibit a complex interaction with their hosts which might be mutualism, antagonism or very rarely parasitism (Nair and Padmavathy, 2014). Endophytes are known to enhance the host growth and nutrient gaining capacity. These microbes improve the plant's ability to tolerate various types of biotic and abiotic stresses as well as enhance the resistance of plants to pathogenic microorganisms, insects and pests. Endophytes can colonize in the stem, roots, petioles, leaf segments, inflorescences of weeds, fruits, buds, seeds as well as dead and hollow plant cells (Hata and Sone, 2008; Specian *et al*., 2012; Stępniewska and Kuzniar, 2013). Endophytic population in a plant species is highly variable and depends on various components, such as host species, host developmental stage, inoculum density and environmental conditions (Dudeja and Giri, 2014). Endophytes are therefore an endosymbiotic group of microorganisms including bacteria (actinomycetes or mycoplasma) or fungi that colonize plants and can be readily isolated from any microbial or plant growth medium.

2.1.1 Endophytic fungi

Among the endophytes, the most relevant and frequently studied group of endophytes is the endophytic fungi. Every plant species in the world is considered to host at least one endophytic fungus within them. Endophytic fungi commonly are reported to include fungal species belonging to Ascomycetes, Deuteromycetes or fungi imperfecti and Basidiomycetes (Petrini, 1986; Dayle *et al*., 2001). However, the number of genera and species of fungi belonging to Ascomycetes and Deuteromycetes are found to be more frequently associated with plants including angiosperms, gymnosperms, bryophytes and pteridophytes as endophytes. It has been estimated that there might be about one million species of endophytic fungi alone (Dreyfuss and Chapela, 1994). Many plants in the natural ecosystem are found to exhibit symbiotic associations with mycorrhizal fungi or fungal endophytes which have a deep effect on the ecology, fitness and evolution of plants (Petrini, 1986; Rodriguez *et al*., 2009). Such symbiotic relationship of fungus has positive effects on the plants and offer qualities like biotic and abiotic stress tolerance, biomass increase, water intake decrease and thus mounting overall health of the plants (Rodriguez *et al*., 2009). Quite a lot of evidences have been found on the fact that endophytic fungi play an important role in host plant physiology. Endophytic fungi receive their nutrition, protection and propagation opportunities from their host plants and on the other hand the host plants also benefit from this symbiosis (Thrower and Lewis, 1973; Clay and Schardl, 2002). They offer protection to the respective host plants from disease causing insects, pests, and herbivores and help their hosts to adapt to stress conditions (Clay *et al*., 2005; Malinowski *et al*., 2005; Knop *et al*., 2007). Endophytic fungi associated with living tissues therefore contribute to the fitness of the plant which makes them different from saprophytic ones. In endophytic association with the plant, the host plant is thought to supply nutrients to the endophytes, while the endophytes might produce factors that protect the host plant from animals, insects and other pathogenic microbes (Yang *et al*., 1994; Huang *et al*., 2001). About 6500 endophytic fungi isolated from different plants were found to have bioactive compounds that aid the host plant to increase their adaptation against various stress conditions (Rodriguez *et al*., 2009). These bioactive compounds produced by the endophytic fungi are reported to be used as antibiotic or antimicrobial and anticancer drugs against pathogenic microbes. The first instance of anticancer drug isolated from endophytic fungi was that of “Taxol” from *Taxomyces andreanae* which supports the secretion of bioactive compounds by endophytic fungi. Other such important bioactive drugs produced by endophytic fungi include penicillins, sordarins, jesterone, clavatol, etc. (Jalgaonwala *et al*., 2011). As such endophytic fungi are considered to have beneficial effects on the host plants as well as are the repositories of novel bioactive compounds that can confer benefit to the humankind against infectious diseases.

2.2 Medicinal plants and endophytic fungi

Studies on fungal endophytes inhabiting medicinal plants have shown that the therapeutic property of the medicinal plant is not only because of the chemicals present in the plant but also because of the fungal endophytes residing present within them (Verma *et al.*, 2011; Suryanarayanan, 2013). At the present times, medicinal plants are found to harbor endophytic fungi having bioactive compounds with high therapeutic properties (Kumar *et al.*, 2005). Currently, there is growing interest to study microbial endophytes harbored within medicinal plants as many of these endophytic microbes have been reported to produce bioactive molecules similar to their respective hosts. Several bioactive metabolites have been reported from endophytic microbes isolated from medicinal plants. As such the endophytic fungal community inhabiting medicinal plants is mostly being investigated all over the globe.

2.2.1 Endophytic fungi from ethno-medicinal plants of India

An increasing surge of interest is recently seen among the research groups for the isolation of endophytes from the tropical plant species owing to high plant diversity (Arnold and Lutzoni, 2007). India being a tropical country having regions of unique ecological niche harbors a variety of medicinal plants. With regard to studies of endophytes (especially fungi) from medicinal plants from India, reports have been found from almost all the high biodiversity regions of India. For instance, from the Nilgiri Biosphere Reserve, Western Ghats, India, 75 dicotyledonous species were sampled to study foliar endophytes and diversity (Suryanarayanan et al., 2011). Also, foliar endophytes from the herbaceous and shrubby medicinal plant species have been studied with emphasis on the colonization rates as well as seasonal diversity in Malnad Region of Bhadra Wildlife Sanctuary in Southern India (Naik *et al.*, 2008; Krishnamurthy *et al.*, 2008). Diversity and host-specificity of endophytic fungal taxa have also been studied from the semi evergreen forest type in Talacauvery subcluster of Western Ghats (Nalini *et al*., 2014). Some ethno-medicinal angiosperm plants of Jammu and Kashmir have also been reported and studied for its major constituents belonging to the steroidal and iridoid family of secondary metabolites, having enormous applications in the medicinal/pharmaceutical fields (Ahmed *et al*., 2012). In the eastern region, endophytic fungal communities associated with two ethno-medicinal plants of Similipal Biosphere Reserve have been studied with emphasis on their antimicrobial prospective (Jena and Tayung, 2013).

2.2.2 Endophytic fungi from ethno-medicinal plants of North-East India

In North-east India, studies on endophytic fungi have been initiated by various workers for their overall diversity and abundance as well as for their beneficial explicabilities towards the mankind. Endophytic fungi from a traditionally important medicinal plant *Litsea cubeba* has been investigated (Deka and Jha, 2017). Another report involves study of fungal endophytes of five medicinal plants prevalent in the traditionally preserved 'Sacred forests' of Meghalaya (Bhagobaty and Joshi, 2012). An ethno-medicinal plant commonly used to treat various ailments in human beings namely *Phrynium capitatum* was studied for endophytic fungal community Papum Pare district of Arunachal Pradesh (Sharma *et al*., 2020). A number of endophytic species were isolated that namely, *Pestalotiopsis longiseta*, *Diplodina microsperma*, *Nodulisporium hinnuleum*, *Aspergillus flavus*, *Diaporthe* sp., *Fusarium incarnatum*, *Nigrospora oryzae*, *Lasiodiplodia viticola*, *Sordaria fimicola*, *Diplodina microsperma*, *Preussia* sp. and

Aureobasidium sp. However, considering the vast range of medicinal plants found to be used by the ethnic communities of Assam, studies on the endophytic colonization of these ethno-medicinal plants are still necessary.

2.3 Endophytic fungi and their bioactive secondary metabolites

Endophytes are found to produce phytohormones and other bioactive compounds of biotechnological interest like enzymes and pharmaceutical drugs (Joseph and Priya, 2011; Parthasarathi *et al*., 2012). They act as pools of novel bioactive secondary metabolites that include alkaloids, phenolic acids, saponins, tannins, quinones, steroids and terpenoids which serve as potential agents with antimicrobial, anti-insect, anticancer and various other bioactive properties. Although plant sources are being expansively explored for new biochemical entities for use in therapeutics, endophytic microbes also constitute an important source for drug discovery (Gouda *et al*., 2016). Since centuries plants have served as a source of medicinal bioactive compounds against numerous disease causing organisms. However, in the recent years, microorganisms associated with plants have proved to offer bioactive products with high therapeutic potential rather than the plants themselves (Subbulakshmi *et al*., 2012). Endophytic microorganisms inhabiting some plants have been found to produce the same secondary metabolites as that of the plant thus making them a promising source of novel compounds. For the past few decades, it has become evident that the discovery rate of active novel chemical entities is declining. In addition to plant sources, endophytic microorganisms play an important role in this search for natural bioactive compounds, with potential use in the health sector and in drug discovery (Lam, 2007). There are over 300,000 higher plant species and it can be assumed that each of the species hosts a complex community of endophytic microbes (Saikkonen *et al*., 1998). Even though the relationship between endophytes and their respective hosts varies within organisms, fungal endophytes form an important part of microbial biodiversity. Some of these endophytes produce important bioactive metabolites for wide therapeutic applications.

Endophytes are rich source of natural products displaying broad spectrum of biological activities. They produce diverse groups of metabolites such as steroids, xanthones, phenols, isocoumarines, perylene derivatives, quinones, furandiones, terpenoids, depsipeptides and cytochalasine, polyketides, alkaloids, peptides, proteins, lipids, shikimates, glycosides, isoprenoids (Mayer *et al*., 2011). Secondary metabolites produced by endophytes inhabiting medicinal plants can be used for curing many diseases since these have been knowingly or unknowingly used by various ethnic communities all over India and the world. Endophytes produce low molecular weight and volatile organic compounds (VOCs) such as alcohols, ketones, esters, acids, and hydrocarbons typically derived from either biosynthetic or degradative pathways (Korpi *et al*., 2009). VOCs attract interest for a variety of potential applications, including use as characteristic markers of fungal growth in indoor environments like workspaces and residential structures as well as volatile antibiotics (Polizzi *et al*., 2012). "Taxol" was the first anti-cancerous drug obtained from *Taxomyces andreanae*, an endophytic fungus of *Taxus brevifolia* (Pacific Yew tree) (Stierle *et al*., 1993). Strobel and Daisy (2003) commented that endophytes could be a goldmine of secondary metabolites. *Pestalotiopsis* sp. can be considered as "the *E. coli* of the rain forests" and *P. microspora*, a "microbial factory" of bioactive secondary metabolites. According to them, a variety of chemical structures are seen such as Taxol, Torreyanic acid and Ambuic acid among others. New organisms and many novel natural products from endophytic fungi might inhibit or kill a wide variety of harmful microorganisms like bacteria, fungi, viruses and protozoans that affect humans and plants. Although endophytes do produce secondary metabolites in culture, but the temperature, composition of the medium and the degree of aeration might affect the amount and type of compounds they produce (Tan and Zou, 2001; Strobel *et al*., 2004). According to recent reviews, the characterization of 138 secondary metabolites from endophytic fungi have reported before 2000 (Tan and Zou, 2001) along with additional 184 metabolites reported by 2006 (Gunatilaka, 2006). Medicinal plants are gaining worldwide attention due to the fact that the herbal drugs are cost effective, easily available and with negligible side effects and medicinal plants harbor endophytic mycoflora. The choice of the plant to be used for exploring endophytes for bioactive metabolites is rather important (Kaul *et al*., 2012). Therefore, medicinal plants which are known to be used since centuries as an alternative source of medicine by ethnic communities are a valuable source of bio-prospecting endophytes.

2.3.1 Antimicrobial metabolites from endophytic fungi of medicinal plants

A significant body of researchers have investigated the secondary metabolites and bioactivity of the endophytic fungi isolated from various medicinal plants. Antimicrobial agents are actually low molecular weight organic natural substances produced by microorganisms which are active at low concentrations against other microorganisms (Guo *et al*., 2008). For instance, endophytic fungus *Phoma* sp. inhabiting *Arisaema erubescens* has been reported to produce a new α-tetralone derivative (3S)-3, 6, 7-trihydroxy- α -tetralone together with cercosporamide, trichodermin and β-sitosterol. These compounds exhibited antifungal and antibacterial activity against a range of pathogenic microorganisms like *Fusarium oxysporum*, *Rhizoctonia solani*, *Magnaporthe* *oryzae*, *Xanthomonas campestris* and *Xanthomonas oryzae* (Wang *et al*., 2012). Likewise, *Phoma* sp. endophytic within *Saurauia scaberrinae* is also known to produce Phomodione that is highly effective against *Staphylococcus aureus*. Terpenoids that include Sesquiterpenes, diterpenoids and triterpenoids produced by endophytic fungi possess antimicrobial activity. A terpenoid compound with known antibacterial activity was obtained from *Phomopsis* sp., an endophyte of *Plumeria acutifolia* (Nithya and Muthumary, 2010). Ethyl acetate extract of *Xylaria* sp. isolated from *Piper aduncum* also are found to produce two novel presilphiperfolane sesquiterpenes with antifungal activity (Silva *et al*., 2010). *Xylaria* sp. from *Ginkgo biloba* reportedly produced 7-amino-4-methylcoumarin which showed antibacterial and antifungal activity against various pathogenic organisms (Liu *et al*., 2008). Another endophytic fungi *Fusarium* sp. produced novel antifungal and antibiotic compounds Fusarielin A, Fusarielin B and Fusarielin C (Kobayashi *et al*., 1995). Endophytic fungus, *F. solani* isolated from *Taxus baccata* produced compounds like 1-tetradecene, 8-octadecanone, 8-pentadecanone, octylcyclohexane and 10-nonadecanone that showed antibacterial as well as antifungal activity (Tayung *et al*., 2011). Endophytic fungus, *Phomopsis* sp. produced three novel metabolites Phomopsin A, B, C and two already known products Cytosporone B and Cytosporone C that possessed antifungal activity (Huang *et al*., 2008). New metabolites Asperfumoid and Asperfumin alongwith other known compounds were isolated from *Aspergillus fumigatus*, an endophyte of *Cynodon dactylon* that actively inhibited *Candida albicans* (Liu *et al*., 2004). Metabolites Pyrrocidines A and B obtained from *Acremonium zeae*, an endophytic fungus of maize displayed significant antifungal activity against *Aspergillus flavus*, *Fusarium verticollioides* and antibacterial activity against most drug resistant gram positive bacteria (Wicklow *et al*., 2005). *Colletotrichum gloeosporioides*, an endophytic fungus in *Artemisia mongolica*, is found to produce a metabolite namely Colletotric acid that displays antimicrobial activity against bacteria as well as against pathogenic fungus, *Helminthsporium sativum* (Zou *et al*., 2000). An antibacterial Naphthaquinone, namely Javanicin that exhibited activity against *Pseudomonas* sp. was isolated from *Chloridium* sp., an endophytic fungus of Neem (Kharwar *et al*., 2008). Therefore, researches during the recent times have led to the discovery of a number of antimicrobial metabolites from endophytic fungi inhabiting medicinal plants.

2.3.2 Anticancer metabolites from endophytic fungi of medicinal plants

Endophytic fungi as a source of anticancer agents have been studied extensively since the isolation of the drug Taxol from the endophytic fungus *Taxomyces andreanae* (Stierle *et al*., 1993). Taxol is a diterpenoid (also known as paclitaxel) that is a very potent anticancer agent isolated for the first time from the bark of the Pacific Yew (*Taxus brevifolia*). Torreyanic acid, a quinone dimer, was reported to be produced from endophyte *Pestalotiopsis microsporum* isolated from *Torreya taxifolia*. Torreyanic acid is a potent cytotoxic agent and found to be 5-10 times more efficient in cell lines that are sensitive to protein kinase C agonists (Lee *et al*., 1996). An alkaloid Camptothecin, a potent antineoplastic agent was also isolated from endophytic fungi *Entrophospora infrequens* inhabiting *Nothapodytes foetida* which is supported by an in vitro cytotoxic assay against human cancer cell lines for lung cancer, liver cancer, and ovarian cancer was performed (Puri *et al*., 2005). Precursors for two clinically useful anticancer drugs (Topotecan and Irinotecan) namely 9-methoxycamptothecin and 10-hydroxycamptothecin were extracted from the endophytic *Fusarium solani* inhabiting *Camptotheca acuminata* (Kusari *et al*., 2009b). Another novel endophytic fungus, *Trametes hirsute* have also been reported to produce Podophyllotoxin and other related aryl tetralin lignans with anticancer potential (Puri *et al*., 2006). Ergoflavin, a novel anticancer agent was isolated from endophytes belonging to family Sapotaceae inhabiting the leaves of an Indian medicinal plant *Mimusops elengi* (Deshmukh *et al*., 2009). Vincristine, an alkaloid with cytotoxic activity mainly used in chemotherapy treatment in acute lymphoblastic leukemia and nephroblastoma was isolated from the endophytic Mycelia sterilia inhabiting *Catharanthus roseus* (Yang *et al*., 2004). Similarly, large number of anticancer agents is reported to be produced by fungal endophytes inhabiting different medicinal plants.

2.3.3 Antioxidant metabolites from endophytic fungi of medicinal plants

Antioxidants are substances that protect cells from the damage caused by unstable molecules known as free radicals. Reactions mediated through free radicals are associated with degenerative diseases like cancer, Alzheimer's disease, etc. As such till date only few antioxidants are approved for clinical applications there is a need to search for new and effective antioxidants. Pestacin and Isopestacin were the first antioxidants isolated from *Pestalotiopsis microspora*, an endophytic fungi associated with *Terminalia morobensis* (Harper *et al*., 2003). A new isobenzofuranone derivative identified as 4,6-dihydroxy-5-methoxy-7-methylphthalide alongwith three other known compounds with antioxidative activity were obtained from an endophytic fungus *Cephalosporium* sp. associated with *Sinarundinaria nitida* (Huang *et al*., 2012). Another antioxidant, Graphislactone A was isolated from *Cephalosporium* sp., an endophytic fungus of *Trachelospermum jasminoides* that was confirmed to have strong antioxidant activity (Song *et al*., 2005). A natural antioxidant Cajaninstilbene acid (CSA), a carboxylic acid, has been reported from *Fusarium*, an endophyte of *Cajanus cajan* (Pigeon pea) (Zhao *et al*., 2012a, b). A new isobenzofuranone derivative 4,6-dihydroxy-5-methoxy-7-methylphthalide alongwith three other known compounds with antioxidative activity were obtained from *Cephalosporium* sp. endophytic in *Sinarundinaria nitida* (Huang *et al*., 2012). Similarly, a strong antioxidant activity was exhibited due to the presence of phenolics and flavonoids by *Xylaria* sp. isolated from *Ginkgo biloba* (Liu *et al*., 2007). The flavonoids and phenolic acid derivatives of the fungus *Chaetomium* sp. from *Nerium oleander* are potential antioxidant resource as they exhibit strong antioxidant activity (Huang *et al*., 2007).

2.3.4 Other bioactivity of endophytic fungi of medicinal plants

Additionally, metabolites from endophytic fungi exhibit various other bioactive properties. Two novel antiviral compounds Cytonic acid A and B have been isolated from *Cytonaema* sp. that are reported to be the novel human cytomegalovirus protease inhibitors (Guo *et al*., 2000). Hinnuliquinone, a potent inhibitor of the HIV-1 protease antiviral compound has been reported from the endophytic fungi inhabiting the leaves of *Quercus coccifera* (Oak trees) (Singh *et al*., 2004). *Diaporthe* sp., an endophytic fungus isolated from leaves of *Pandanus amaryllifolius* produced two new benzopyranones: Diaportheone A and B, both of which inhibited the growth of virulent strains of *Mycobacterium tuberculosis* (Bungihan *et al*., 2011). A number of immunomodulatory compounds have been reportedly isolated from endophytic fungi. Two important immunosuppressive compounds: Subglutinol-A and Subglutinol-B were isolated from the fungal endophyte, *Fusarium subglutinans*, inhabiting within *Tripterygium wilfordii* (Lee *et al*., 1995). Another potent immunosuppressive fungal metabolite Mycophenolic acid used for the treatment of autoimmune diseases and organ transplantations has been reported to be produced by endophytic *Penicillium, Aspergillus, Byssochlamys* and *Septoria* species (Bentley, 2000; Larsen *et al*., 2005). Several endophytes are also known to have insecticidal properties. Naphthalene that has insect repellent activity was produced by fungus *Muscodor vitigenus* which was isolated from *Paullina paullinoides* (Daisy *et al*., 2002a, b). An endophytic fungus, *Phomopsis archeri* of *Vanilla albindia* produces aromatic sesquiterpenesphomoarcherins A–C which show antimalarial activity against *Plasmodium falciparum* (Hemtasin *et al*., 2011). Thus, endophytic fungi from medicinal plants are found to be a novel and important microbial resource for producing bioactive compounds with potential applications. Plethora of bioactive metabolites produced by endophytic fungi inhabiting plants are indeed a hidden treasure worth exploring.