CHAPTER- I
INTRODUCTION

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Microorganisms form an integral part of any biotic as well as abiotic systems and their diversity depends on the different kinds of habitats that they colonize. The reason why life sustains on earth is because of their nutrient recycling and maintaining the function of the ecosystem. They are prolific producers of enzymes and secondary metabolites and have wide biotechnological applications, therefore considered invisible bio-resource of any nation. They can be parasitic, free-living, symbiotic or can obtain nutrition from different ways. Most of the microbial associations with biotic systems, either plants or animals have mostly been studied for their pathogenicity against the respective hosts. However, there are also reports that encompass the beneficial properties of microbial interactions. One such beneficial plant-microbe association are the endophytes, which are basically endo-symbiotic group of microorganisms that colonize inner plant tissues without causing any disease symptoms. A definition of an endophyte was provided by De Bary (1866) for the first time as "*an organism that grows within plant tissues without causing any disease*". However, the definition varies among various researchers and later on Petrini (1991) provided the most suitable definition for endophytes, which states "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 are said to be endophytes". Endophytic microbes are associated with plants in various forms, including bacteria (actinomycetes or mycoplasma) or fungi that colonize inner plant tissues without overt symptoms. The most commonly isolated endophytes and studied groups are the fungi.

Fungal endophytes were first reported in grasses and trees like *Picea canadiensis* (Lewis, 1924). Since then, endophytic fungi have been reported for every plant species investigated so far from all possible plant parts like leaves, stem, roots, seeds, fruits, bark and vascular bundles. They mostly reside in the internal tissues of roots, stems, leaves, flowers, and seeds (Bacon and White, 2000). The endophytes may be transferred either horizontally or vertically (Hartley and Gange, 2009). Every plant species in the world is considered to be associated with at least one endophytic microbe. Among them, fungi are dominantly isolated as endophytes and their associations have been reported from every plant species studied so far. Fungi are thus common as endophytes and show high diversified presence within plant tissues (Faeth and Fagan, 2002). Endophytic microbes are considered as repositories of bioactive metabolites and serve as an outstanding source of drugs for treatment of various infectious diseases (Strobel and Daisy, 2003; Shukla *et al*., 2014). They act as reservoirs of novel bioactive secondary metabolites that serve as a potential candidate for antimicrobial, anti-insect, anticancer properties and many wider therapeutic applications (Gouda *et al*., 2016). Endophytic fungi produce some of the most broadly used antibiotic and anticancer drugs. For instance, Taxol, isolated for the first time from *Taxomyces andreanae*, is the most effective and successful anticancer drug extracted from endophytic fungi so far (Zhang *et al*., 2009). Since then, various drugs used in the medical industry have been sourced from endophytic fungi. Drugs like Penicillenols extracted from *Penicillium* sp., is cytotoxic to numerous cell lines. Other important drugs isolated includes Clavatol from *Torreya mairei*, Jesterone from *Pestalotiopsis jesteri*, Sordaricin from *Fusarium* sp. and Javanicin from *Chloridium* sp. that possess strong antibacterial and antifungal properties against a range of infectious agents (Jalgaonwala *et al*., 2011). Another important drug with anti-mycotic and

antioxidant properties, namely, Pestacin was isolated from *Pestalotiopsis microspora* (Harper *et al*., 2003).

Over the recent years, there has been an enormous increase in instances of antimicrobial drug resistance of pathogenic microorganisms towards the existing drugs which has emerged as a global health concern. This has threatened the effective prevention as well as treatment of the increasing infections caused by various bacteria, fungi and viruses. Amongst important health problems, multidrug resistance pathogenic bacteria and invasive fungal infections have been reported to be a serious public hazard, especially for the increasing number of immune-compromised populations including patients suffering from AIDS or cancer as well as those under organ transplantation and in intensive care units (Rabkin *et al*., 2000). The prevalence of bacterial and invasive fungal infections has increased significantly in the recent times threatening the world community. Therefore, there is an urgent need for new and effective therapeutic agents to provide assistance to ever growing human health problems of the world such as drug resistance in bacteria, the appearance of life-threading viruses, increased incidence of fungal infections and new effective anticancer and anti-plasmodial agents. However, only a limited number of antimicrobial agents are currently available for the treatment of life-threatening diseases caused by pathogenic bacteria and fungi. Although new antimicrobial agents have been introduced in the pharmaceutical market, the development of resistance to these drugs has become increasingly apparent, especially in patients with long term treatment. However, in comparison to the more rapidly developing multidrug resistant microorganisms, the discovery and development of new antimicrobial agents are decreasing. Thus there is a general call for new antibiotics and chemotherapeutic agents that are highly effective possess low toxicity and have minor environmental impact.

Microorganisms are considered as the richest source of antibiotics. Since the discovery of "Penicillin" from *Penicillium,* the search for new and effective antimicrobial agents from microbes are underway. Realizing their potential, research priorities are directed towards exploration and discovery of antimicrobial agents from new biological niches of microorganisms. In such a scenario, "endophytes" that colonize healthy plant tissues can be considered as a resource for discovering new and interesting metabolites since many novel and effective bioactive metabolites have been reported from them (Strobel *et al*., 2003; Staniek *et al*., 2008; Shukla *et al*., 2014).

In recent years, search for bioactive metabolites have been directed towards fungal endophytes colonizing medicinal plants because of the facts that these microbes have produced similar bioactive natural products as their respective host and sometimes displaying higher bioactivity. Medicinal plants and their endophytes has been found to be an important source of precious bioactive compounds and secondary metabolites that contribute to more than 80% of the natural drugs available in the market (Singh and Dubey, 2015). Of late, endophytic fungi isolated from medicinal plants have received significant attention due to isolation of many new and interesting bioactive metabolites with wide therapeutic applications (Joseph and Priya, 2011; Zhao *et al*., 2011a). The isolation of the most promising anticancer agent, "Taxol" from the endophytic fungus, *Taxomyces andreanae* from bark of the medicinal plant *Taxus brevifolia* has provided an unconventional approach to attain a cheaper, effective and ecofriendly product from endophytic microorganisms (Stierle *et al*., 1993). Medicinal plants are often used by marginal communities to treat various ailments all around the world. It is now known that such plants harbor large number of endophytic fungi with potential for tapping bioactive metabolites. India being one of the mega diversity countries in the world is a home to a

large number of medicinal plants but only few of them have been explored for endophytic microbes. The North-Eastern region has been identified as the Indo-Burma biodiversity hotspots and home to a number of endemic and ethno-medicinal plants. Assam, one of the states of North East Region (NER) of India is a rich hub of medicinal and aromatic plants. This region is also well-known for diverse culture of human races and is also home to a large number of ethnic tribal communities. These tribes possess, through generations of ethno-pharmacological experience, a vast traditional knowledge on effective herbal medicines which is usually passed on by oral traditions as a guarded secret of certain families. The local ethnic tribal communities of Assam use these plants in culinary or in other ways to treat various ailments. However, these plant resources are less studied in terms of the endophytic fungi and metabolites they produced still need to explore and elucidated. Furthermore, this region is also believed to have enormous microbial diversity which is understudied and their exploration might have applications in agriculture, nutrient management and industry. Thus considering the myriads of medicinal plants of this region and only few of them have been investigated for fungal endophytes, so there is every possibility of discovering novel bioactive metabolites from endophytes associated with these plant resources.

Strobel and Daisy (2003) suggested that areas of high biodiversity and with high numbers of endemic plant species may hold the most potential for endophytes with novel chemistry. Ethno-medicinal plants of a highly bio-diverse region like Assam are likely to host a large collection of microbial endophytic community with novel metabolites but still less explored. Therefore, there is a great chance of obtaining potent endophytic fungal strains inhabiting these plants that might produce novel metabolites with wide therapeutic applications. Considering the importance of medicinal plants and their associated

endophytic fungi as prolific producer of bioactive metabolites, the present work is proposed to study the endophytic fungi associated with some selected ethno-medicinal plants of Assam with special reference to their antimicrobial metabolites.

Therefore, keeping in view the above particulars, the present study was conceptualized with the following objectives:

1. Isolation and identification of endophytic fungi from some selected ethno-medicinal plants.

2. Screening of endophytic fungal isolates for antimicrobial activities against some human test pathogens.

3. Optimization for enhanced metabolite production by some potent isolates under different cultural conditions.

4. Purification and characterization of metabolites produced from some potent endophytic fungi.