GENERAL INTRODUCTION

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, many based on their usage of traditional medicine. Drug derived from natural sources play a significant role in the prevention and treatment of human diseases. About 61 percentage of new drug development between 1981 and 2002 were based on natural products and they have been very successful especially in the area of infectious diseases and cancer (Cragg and Newman, 2005). More than 90 percentage of the terms recorded in Indian medicinal literature are derived from plant sources (Joseph and Priya, 2010). Recent trends, however, show that the discovery rate of active novel chemical entities is declining due to the habitat destruction, over exploitation of medicinal plants by pharmaceutical companies and urbanization due to the explosive population in need of food and shelter (Lam, 2007). Therefore, there is an alternative need for the resurgence in the consumption and demand for medicinal plants, especially through endophytes.

The term endophyte was coined by the German scientist Heinrich Anton De Bary, (1866). Endophytes are microorganisms that include bacteria, fungi and actinomycetes living within plant tissues without causing any immediate overt negative effects. Endophytes are ubiquitous with rich biodiversity. They have been found in every plant species examined to date and recognized as the potential sources of novel natural products for exploitation in medicine, agriculture and industry with more bioactive natural products (Bacon and White, 2000). It is noteworthy that nearly 3,00,000 plant species exist on the earth and each individual plant is host to one or more endophytes (Strobel and Daisy, 2003). Microorganisms are important sources of bioactive natural products with enormous potential for the discovery of new molecules for drug discovery,
industrial use and agricultural applications (Demain, 1999; Keller et al., 2005; Strobel, 2004; Porras-Alfaro and Bayman, 2011).

Endophytes are metabolically more active than their free counterparts due to their specific functions in nature and activation of various metabolic pathways to survive in the host tissues (Strobel and Daisy, 2003; Strobel, 2006; Riyaz-Ul-Hassan et al., 2012). Many endophytes have the potential to synthesize various bioactive metabolites that may directly or indirectly be used as therapeutic agents against numerous diseases (Strobel et al., 2004; Staniek et al., 2008; Aly et al., 2010; Kharwar et al., 2011; Kusari and Spiteller, 2012b). Occasionally, endophytes that produce host plant secondary metabolites with therapeutic value or potential have been discovered; some examples include paclitaxel (also known as Taxol) (Stierle et al., 1993), podophyllotoxin (Eyberger et al., 2006; Puri et al., 2006), deoxypodophyllotoxin (Kusari et al., 2009a), camptothecin and structural analogs (Puri et al., 2005; Kusari et al., 2009c, 2011b; Shweta et al., 2010), hypericin and emodin (Kusari et al., 2008, 2009b) and azadirachtin (Kusari et al., 2012).

Fungi are known to produce pharmaceutically important compounds like penicillin, cephalosporin, steroids, hormones, citric acid, cheese products and enzymes for commercial products. Fungal endophytes have been detected in hundreds of plants, including many important agricultural commodities such as wheat (Larran et al., 2002a), bananas (Cao et al., 2002), soybeans (Larran et al., 2002b) and tomatoes (Larran et al., 2001). Several roles have been ascribed to fungal endophytes, including providing protection against herbivorous insects (Breen, 1994; Clement et al., 1994), plant parasitic nematodes (West et al., 1988; Elmi et al., 2000) and plant pathogens (White and Cole, 1986; Dingle and McGee, 2003; Wicklow et al., 2005).

Actinomycetes are also well known as producers of antibiotics and other biologically active substances with high commercial value, such as vitamins, alkaloids, plant growth factors, enzymes, and enzyme inhibitors (Tanaka and Omura, 1990). Actinomycetes are also found to colonize the plant tissues as endophytes. Endophytic Actinomycetes have been isolated from
surface sterilized roots of Italian native plants (Sardi et al., 1992), from roots and leaves of maize (De-Araujo et al., 2000), from roots and leaves of banana plants (Cao et al., 2004) and from surface sterilized wheat roots (Coombs and Franco, 2003). The role of the endophytic actinomycetes to improve the plant growth and it protects from plant pathogens have also been reported (Tokala et al., 2002; Taechowisan et al., 2003; Tian et al., 2004).

**Bio active compounds from endophytic fungi**

Endophytic fungi are also believed to be a potential source of novel bioactive compounds, such as antibiotics, antiviral, anticancer agents, antioxidants, insecticidal and immunomodulatory (Tan and Zou, 2001). *Fusarium* sp. have been reported as endophytes from several plants with diverse biological activity (Shiono et al., 2007; Kour et al., 2008; Deng et al., 2009). This suggests their ubiquity as endophytes within the plant kingdom and provides an opportunity to discover novel bioactive metabolites. *Fusarium solani* has been reported as an endophyte from several Yew species with production of the anticancer metabolite taxol *in vitro* (Chakravarthi et al., 2008; Li et al., 2005). Endophytic fungi are also capable to produce antimicrobial metabolites. Cryptocandin, a unique peptide, isolated from the fungus *Cryptosporiopsis quercina* which is an endophyte of *Tripterigeum wilfordii* (Strobel et al., 1999). Cryptocin, a unique tetramic acid, isolated from *Cryptosporiopsis cf quercina* reported to be active against plant pathogenic fungi *Pyricularia oryzae* (Li et al., 2000) likewise phomopsichalasin was isolated from *Phomopsis cassia*, an endophyte in *Cassia spectabilis* (Silva et al., 2005).

There are limited compounds reported as antiviral agents from endophytic fungi. Two novel compounds cytonic acid A and B have been isolated from the endophytic fungus *Cytonaema sp.* reported as inhibitor of human Cytomegalovirus (hCMV) protease (Guo et al., 2000). Pestacin and isopestacin, which were isolated from *Pestalotiopsis microspora*, have the antioxidant property isolated from *Terminalia moobensis*, native of the Papua New Guinea (Strobel et al., 2002; Harper et al., 2003). The endophytic fungi are well known for their role to
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protect host from the insects and pests. It is believed that ergot alkaloids and mycotoxin are mainly responsible for their insecticidal activity. Peramine is a pyrolopyrzine alkaloid which is an insecticide but harmless to mammals was isolated from fungi *Neotyphodium coenophialum, Neotyphodium loli*, *Epichloe festucae* and *Epichloe typhina* present in the stem and leaf of tall fescue, rye grass and other grasses. Naphthalene, that has insect repellent activity, is produced by fungus *Muscodor vitigenus* which was isolated from liana (*Paullina paullinioides*) (Daisy et al., 2002). Immunosuppressive compounds pyrones subglutinols A and B were isolated from the endophyte *Fusarium subglutinans* isolated from *Tripterygium wilfordii*. Pyrones subglutinols A and B are nontoxic and very potent in the mixed lymphocyte reaction (MLR) and thymocyte proliferation (TP) assays (Lee et al., 1995).

**Bioactive compounds from endophytic Actinomycetes**

Drug discovery point of view, the novel actinobacteria strains are attractive, as they are likely to contain new genes in theory and held promising for novel products, thus there is a chance of finding novel pharmaceutical bioactive compounds from endophytic actinobacteria. Endophytic actinobacteria associated with traditionally used medicinal plants especially of the tropics could be a rich source of functional metabolites (Strobel et al., 2004). Antibiotics from endophytic actinobacteria, especially those from medicinal plants possess the ability of inhibiting or killing a wide variety of harmful microorganisms like pathogenic bacteria, fungi and viruses. Thus, there is a great application value to develop antimicrobial drugs from endophytic actinobacteria. A lot of new antibiotics have been isolated from endophytic actinomycetes, such as Munumbicins A-D (Castillo et al., 2002), Celastramycin A-B (Pullen et al., 2002), Kakadumycins (Castillo et al., 2003), Demethylnovobiocins (Igarashi, 2004), Cedarmycin A and B, Actinomycin D (Taechowisan et al., 2006), Saadamycin (El-Gendy and EL-Bondkly, 2010), Anthraquinones, Lupinacidins 50 A and B (Igarashi et al., 2007), Pterocidin (Igarashi et al., 2006), 6-alkylsalicylic acids, Salaceyins A and B (Kim et al., 2006). Recently, two compounds 5,
7-dimethoxy-4-phenylcou-50marin and 5, 7-dimethoxy-4-p-methoxylphenylcoumarin, originally produced by numerous species of plants were isolated from endophytic *Streptomyces aureofaciens* to have antifungal, anti inflammatory and antitumor activity (Taechowisan et al., 2005, 2007a, 2007b). Therefore, the search for novel compounds could be directed towards plants that commonly serve indigenous populations for medicinal purposes as they are expected to harbour novel endophytes that may produce unique metabolites with diversified applications (Strobel and Daisy, 2003).

The Solanaceae or nightshades are an economically important family. The family belongs to the order Solanales, in the asteroid group dicotyledons (Magnoliopsida) (Olmstead et al., 1999). The family includes a number of important agricultural crops, medicinal plants and ornamentals. Many members of the family contain potent alkaloids which have an intense physiological action. As far as human concerned, these alkaloids can be desirable and toxic or both. The tropanes are the most well-known of the alkaloids that are found in the solanaceae (such as scopolamine, atropine and hyoscyamine). The plants that contain these substances have been used for centauries as poisons. However, despite being recognized as a poison many of these substances have invaluable pharmaceutical properties. Pharmacologically, they are the most powerful known anti-cholinergics in existence, meaning that they inhibit the neurological signals transmitted by the endogenous neurotransmitter, acetylcholine. More commonly, they can halt many types of allergic reactions. Symptoms of overdose may include dry mouth, dilated pupils, ataxia, urinary retention, hallucinations, convulsions, coma, and death. Atropine, as tropane alkaloids is commonly used as ophthalmological agent it dilates the pupils and thus facilitates examination of the interior of the eye also a stimulant effect on the central nervous system and heart, whereas scopolamine has a sedative effect. Despite the extreme toxicity of the tropanes, they are useful drugs when administered in extremely small dosages. They can reverse cholinergic poisoning, which can be caused by over exposure to organophosphate insecticides and chemical
warfare agents. Scopolamine (found in *Hyoscyamus niger* and *Scopolia atropioides*) is used as an antiemetic against motion sickness or for people suffering from nausea as a result of receiving chemotherapy (Evans, 1979). Scopolamine and hyoscyamine are the most widely used tropane alkaloids in pharmacology and medicine due to their effects on the parasympathetic nervous system. These alkaloids cannot be substituted by any other class of compounds and for this reason they are still in demand. This is one of the reasons for the development of an active field of research into the metabolism of the alkaloids, the enzymes involved and the genes that produce them. Hyoscyamine 6-β hydroxylase, catalyses the hydroxylation of hyoscyamine that leads to the production of scopolamine at the end of the tropane’s biosynthetic pathway. This enzyme has been isolated and the corresponding gene cloned from three species: *Hyoscyamus niger*, *Atropa belladonna* and *Brugmansia candida* (Matsuda et al., 1991; Rocha et al., 2002). Hence the present study was aimed to isolate endophytes from the selected medicinal plants of solanaceae members and screening them for antibiological activities such as antimicrobial activities, α-glucosidase inhibition and antioxidant activity followed by optimization, purification of active compounds and tested for anticancer activities.