CHAPTER 2 REVIEW OF LITERATURE

The importance of fungi for production of bioactive compounds was initially apprehended by the discovery of penicillin from *Penicillium notatum*, by Alexander Fleming in 1928. After that, considerable attention toward discovery of natural products from fungi has been focused and drives, especially after the large-scale production of penicillin during World War II. In general, microorganisms themselves represent as promising sources of natural products having the advantage of production of sustainable and sizeable quantities of bioactive compounds by large-scale cultivation and fermentation of the source organisms with reasonable cost (Waites et al., 2001). In earlier time micro fungi were isolated mainly from soil samples, but in the search for new sources of bioactive metabolites especially those that are associated with higher plants and these fungi have been proved to be a potential source of bioactive metabolites producing a vast category of new biologically active secondary metabolites (Aly et al., 2010; Bugni TS, Ireland CM 2004; Debbab et al., 2010; Xu et al., 2010; Blunt et al., 2011; Rateb and Ebel, 2011).

In recent time bioprospecting is a term that is generally used to give explanation about the search of bioactive metabolites or organisms of commercial importance from the biological community of world. The reality lies in the fact that tropical forests are more species-rich than temperate forests, or arid forests and that surrounded by tropical regions have extreme plant and microbial diversity. Therefore, sampling of a particular group of fungi of a habitat in a defined area will be more revealing and fruitful in search of the undescribed fungi for natural products (Rodriguez et al., 2001). It has been estimated approximately about 300,000 species of vascular plants which works as a reservoir of a large numbers of microbes in their tissue known as endophytes Promputtha et al., 2007). The term ‘endophyte’ includes all

organisms that grow inside plant tissues without causing disease symptoms (Petrini, 1991 and Chanway, 1996). Endophytic microorganisms are therefore may be defined as those microorganisms (mostly fungi and bacteria) which spend their life in the plant tissues generally bellow the epidermal cell layers without causing any disease symptoms to the host plant (Strobel and Daisy, 2003). Under certain conditions endophytes may become parasitic, and become pathogens causing symptomatic infection (Brown et al., 1998). It can be regarded as an unbalanced status of a symbio-sis when the host is stressed and physiological or ecological conditions favors virulence (Müller et al., 2005, Schulz & Boyle 2005, Kogel et al., 2006). Endophytes of certain plant could be a pathogen of other plants, depending on the balance between pathogenicity and endophytism of the microorganism in the different hosts (Saikkonen et al., 2004). Once the host-endophyte interaction becomes imbalanced either disease results in the host plant or the plant defense machinery kills the pathogenic endophytic fungus. Whether the interaction is balanced or imbalanced depends on the general status of the partners. The virulence of the fungus, and the defenses of the host, are being variable and influenced by environmental factors, nutritional status and developmental stages of the partners. Hence, commensalism and mutualism require a sophisticated balance between the defense responses of the plant and the nutrient demand of the endophyte (Kogel et al., 2006).

Plant endophytic fungi, have the special ability to produce the same or similar compounds originated from their host plants, as well as a great number of diverse bioactive compounds, which have been implicated in the protection of its host against pathogens and herbivores (Wicklow et al., 2005, Strobel and Daisy 2003; Strobel, G. 2003; Yuan et al., 2008; Suryanarayanan et al., 2009). One of the best examples productions of same kind of

metabolite is the production of gibberellins in *Fusarium fujikuroi* Nirenberg in the early 1930s. In due course it was determined that the gibberellins are one of only five classes of phytohormones that are to befound in virtually all plants (Stierle et al., 1993).

The role of natural products in discovery of new therapeutic agents can be demonstrated by an analysis of the number and sources of bioactive agents. There are at least 200,000 natural metabolites with various bioactive properties (Berdy, 2005). The discovery of natural products involves isolation, structural elucidation and establishing the bio-synthetic pathway of the secondary metabolites. This is an area of considerable interest to scientists due to the structural diversity, complexity and various bioactivities of isolated compounds (Selim et al., 2001). World's first billion and one of the most famous and the anticancer drug, taxol, was firstly isolated from the Pacific yew tree in the early 1960s and the drug has been approved for the treatment of ovarian cancer by the U.S. Food and Drug Administration in 1992. This drug was also found effective against the lung, breast, head and neck cancer, and also in the advanced forms of Kaposi's sarcoma (in people with AIDS). Taxol (mitotic inhibitor) has a unique mechanism of action of breakdown of microtubule during cell division (Horwitz S.B., 1993). Besides its clinical application, taxol also has its application as microtubule stabilizer in biological and biomedical research. Taxol is found extremely in low amounts in the needles, bark, and roots of Taxus spp. This has enforced to the search for novel sources of taxol from trees and isolation of an endophytic fungus (Yuan et al., 2006, Strobel GA, 1996). So far, there are about more than 30 species isolated from plants producing taxol. However, the separation of endophytic fungi from plant materials is comparatively a simple process, but the identification of taxol-producing endophytic fungus is difficult (Zhou et al., 2007). .As an alternative method, genes encoding for taxol biosynthetic enzymes have been used as

molecular marker for screening taxol producing fungal endophytes. Another surprising fact in reference to drug resistance is that there is decrease in number of receiving approval of new antibacterial drugs over the past few years. After the search of first endophytic fungus, a lot of attention has been focused on potential of exploitation of these fungi for the production of novel bioactive metabolites therefore this biological community should be thoroughly investigated and used as a base for sustainable research and development of new antibacterial substances that can both respond to current antimicrobial resistance and anticipate evolving resistance (Gunatilaka et al., 2006; Guo et al., 2008).

Biologically active secondary metabolites from marine-derived fungi have also proven to be of great importance (Bugni and Ireland, 2004). The finding of new species of endophytic fungi and the search of novel antibacterial substances should continue. The reasoning behind host plant selection has mostly been investigated and plants that are used in traditional medicine for the treatment of infections harbor endophytic fungi in different parts of those plants. Bioassay-guided fractionation and purification procedures play major role in the success of discovering naturally occurring therapeutic agents. Fractionation of the culture broth and mycelium extract leads to the isolation of the metabolite responsible for the antibacterial activity. In the following text we have compiled some review results of antibacterial testing of crude extracts and purified substances obtained from a variety of endophytic fungi, source plant from which they were isolated, the host plant's habitat, type of extract or secondary metabolite, strains of human pathogenic bacteria with antibacterial activity, and the method used for antibacterial testing and the literature references (Table 1, Natasa et al., 2012). In addition to this, many endophytic fungi have been reported to produce valuable secondary metabolites with anticancer, antioxidant and anti protozoal and

other biological activities (Rangarajulu, 2010). Anti-infective fungal metabolites, two new azaphilone derivatives, penicilazaphilones A (1) and B (2), and one new isocoumarin, penicilisorin (3), along with six known compounds were isolated from an endophytic fungal strain obtained from leaves of Garcinia atroviridis (Guttiferae), collected in Yala Province, Thailand. Compound 2, the known sclerotiorin (4), and 2,4-dihydroxy-6-(5,7S-dimethyl-2-oxo-trans-3-trans-5-nonadienyl)-3-methylbenzaldehyde (5) were evaluated for their inhibitory effect on human immuno-deficiency virus HIV-1 integrase and protease, as well as for their anti-microbial activity against *Staphylococcus aureus*, *Candida albicans* and *Cryptococcus neoformans* (Arunpanichlert et al., 2010). Mostly the groups of fungi which come under the term of endophytic are filaments fungi, which extend through the plant tissue where the fungus grows (Sieber, et al., 1990).

The symbiosis relationship between plant and endophyte was determined, namely, plant part protects and feeds the latter while fungal counterpart produces bioactive substance namely, plant growth regulatory, antibacterial, antifungal, antiviral and insecticidal,to enhance the growth and competitiveness of the host plant in the community (G.C. Carroll, 1998). Accordingly, some endophytes could be reliable sources of secondary metabolites which may play an important role in agricultural and pharmaceutical fields and it is confirmed by production of taxol (A. Stierle et al., 1993) and other such as antibacterial (Marica et al., 2004), antifungal (Garcia et al., 2012), anticancer (Chandra et. al., 2012), antiprotozoal (Gao et al., 2012), antioxidant (Yang et al., 2007) antiviral (Zhang et al., 2006), antimalarial (Lu et al., 2000), antitubercular (Yu et al., 20010), immunosuppressive, antidiabetic and antiviral (Zhang et al., 2013). These bioactive compounds could be mainly classified as Alkaloids, steroids, terpenoids, quinones, phenylpropanoids, isocoumarins, lignans, phenols and

lactones etc (Zhao et al., 2010). These bioactive compounds used for the healthcare purpose for human beings (Guo et al., 2007). Several natural products produced by endophytic fungi have unique structures and large bioactivities which applied in agricultural, industrial and medicinal fields (Anderson et al., 2011; Nitya et al., 2011). Approaches for development of new antibiotics have been pursued, such as combinatory chemistry tools but only a few broad spectrum antibiotics are reported to be produced by the pharmaceutical industry at the present time (Coates et al., 2012). The pathway of production of an array of metabolites by endophytic fungi have also been studied and it has been observed that they are produced from different biosynthetic pathways,including polyketide, isoprenoid and amino acid and belong to diverse structural groups,such as terpenoids, steroids, xanthones, quinones, phenols, isocoumarins, benzopyranones,tetralones, cytochalasins and enniatins (Schulz et al., 2002).

2.1 Antibacterial activity

Large number of antibacterial substances has been isolated from endophytic fungi. It is generally observed that the number of secondary metabolites produced by endophytic fungi is larger than that of any other endophytic microorganism class. The partial possible explanation behind this could be a consequence of the high frequency of isolation of endophytic fungi from plants (Zhang et al., 2006). Due to the same reason, some fungal genera seem to have a higher frequency of isolation and therefore a relatively greater chance of discovering an antibacterial substance produced by its belongings (Table 1).

Table1. Endophytic fungi producing metabolites with antibacterial activity

Endophytic fungal strain	**Host plant(s) (family), plant part or tissue**	**Crude extract/isolated metabolite**	**Test bacteria**	**Type of test**	**Reference**
Phomopsis isolate MF6031	Salix gracilistylavar. Melanostachys (Salicaceae); twig	Phomopsichalasin	B. subtilis E.faecium P.aeruginosa S. aureus	Disk diffusion assay	Horn et al., 1995
Colletotrichum gloeosporioides, Alternariaalternata, Guignardiabidwelli, Phomopsisarcheri and *Drechslera dematioidea*	LippiasidoidesCham. (Verbenaceae); Leaves and stems	Fungal mycelium	S. aureus (ATCC-6538), B. subtilis (UFPEDA-16), E. coli (ATCC-25922).	Antimicrobial assay using a solid medium	Ichikawa et al.,1971 De Siqueira et al., 2011
Acremoniumsp., Diaporthesp., Hypoxylonsp., Pestalotiopsis sp., *Phomopsissp., Xylaria sp.,* and other	Aegicerascornic ulatum, Avicennia alba, Avicennia officinalis, Bruguieragymno rrhiza, Bruguieraparvifl ora, Lumnitzeralittor ea, Rhizophoraapicu lata, Rhizophoramucr onata, Sonneratiacaseol aris Scyphiphora hydrophyllacea, Xylocarpusgrana tum and Xylocarpus moluccensis; leaves and branches	Ethyl acetate crude extract of culture medium Ethyl acetate and hexane extract of fungal mycelium	S. aureus ATCC25923 A clinical isolate of MRSA SK1, E. coli ATCC25922P.aeruginosa ATCC27853	microdilution method according to a modification of Clinical and Laboratory Standards Institute (CLSI) M7-A4	Buatong et al., 2011

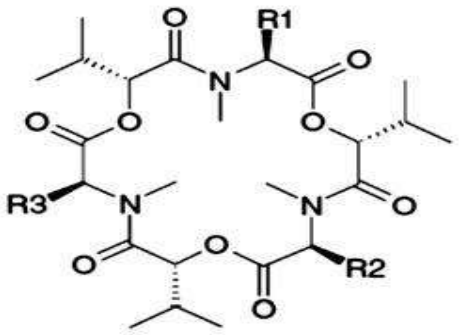
Guignardiasp.	Spondiasmombi nL. (Anacardiaceae);	Ethyl acetate crude	extract of culture broth S. aureus E. coli	Microtiter plate assay)	Rodrigues et al., 2000
Colletotrichu m gloeosporioid es	Artemisia mongolica (Fisch. ex Bess.) Nakai (Asteraceae); stem	Colletotric acid	B. subtilis S. aureus Rosenbach Sarcinalutea SchroeterPseudomonas sp.	Paper-disk assay on	LB Zou et al., 2000
13 isolates of *Phomopsis sp.*	Aspidosperma tomentosumMA RT. (Apocynaceae); leaf Spondiasmombi nL. (Anacardiaceae); twig	Ethyl acetate crude extracts of cultivation broth	*E. coli* (ATCC 25922) *P. aeruginosa* (ATCC 27853) S. aureus (ATCC 25923)	agar-overlay assay	Corrado and Rodrigues, 2004
Phoma sp. NG-25	Saurauiascaberri nae (Actinidiaceae); stem Central highlands	Phomodione Usnic acid Cercosporamide	*S. aureus* (ATCC 25923) *E. coli* (Life Technology 18290–015)	Disk diffusion assay	Hoffman et al., 2008
Rhizoctonia sp. strain Cy064	Taxus cuspidate Siebold &Zucc (Taxaceae); small branch	Periconicin A Periconicin B	*E. coli* (ATCC 25922) *K. pneumoniae*(IFO 13541) *P. vulgaris* (ATCC 3851),*S. typhimurium*(ATCC 14028),*B. subtilis* (ATCC 6633) *M. leuteus*(IFO 12708)S. aureus (ATCC 6538p) S. epidermis (ATCC 12228) Twofold	microtiter broth dilution method	Kim et al., 2004
Fusarium sp. IFB-121	Quercusvariabili s Blume (Fagaceae); bark	Cerebroside 1 Cerebroside 2	*B. subtilis* *E. coli*	Liquid dilution method	Shu et al., 2004
Xylaria sp. YX-28	Ginko biloba L. (Ginkgoaceae); twig	7-Amino-4-methylcoumarin	*S. aureus,E. coli* *S. typhi,S. typhimurium* *S. enteritidis ,Aeromona shydrophila, Yersinia sp. V.anguillarumShigellasp.*	dilutions method	Liu et al., 2008

Pichia guilliermondii Ppf9	Paris polyphyllavar. yunnanensis(Fra nch) Hand.-Mazz. (Trilliaceae);	Helvolic acid	E. *coli* (ATCC 29425), *B. subtilis* (ATCC 11562), *S. aureus* (ATCC 6538) *S. haemolyticus*(ATCC 29970)	A modified microdilution-colorimetric assay, using the chromogenic MTT reagent (Zhao and Zhou (2008) Zhao et al. (2010	
Alternariasp. strain JCM9.2	Sonneratia alba J.E. Smith (Sonneratiaceae)	• Xanalteric acid I • Xanalteric acid II • Altenusin	*E. coli* *E. faeciumE.cloacae* *S. aureus* *S. pneumonia* *P. aeruginosa* *K. pneumonia*	Dilution assay	Kjer et al., 2009
Microdiplodia sp. strain 7092	Erica arboreaL. (Ericaceae); ns	3,4-Dihydroglobosuxanth one A	*E. coli*	Agar diffusion assay	Krohn et al., 2009
Microsphaero psissp. strain 7177	Zygophyllumfort anesii (Zygophyllaceae)	Fusidienol A 8-Hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid methyl ester	*E. coli*	Agar diffusion assay	Krohn et al., 2009
Trichoderma ovalisporumP RE-5	Panaxnotoginsen g (Burkill) F.H.	Koninginin A (E)-2,3-dihydroxypropyl octadec-9-enoate Shikimic acid Cytosine ribonucleoside A compound	S. *aureus* *B. cereus* *M. luteus* *E. coli*	Paper-disc diffusion method	Dang et al., 2010

2.2 Antifungal activity:

A variety of antifungal metabolites from different endophytic fungal source have been extracted and reported to show antifungal activity against a variety of pathogenic fungi, (Hong Lu et al., 2000) including activities to the crop pathogenic fungi; *Phytophthora Capisici, Rhizoctonia cerealis, Gaeumannomyces graminis var. tritici and Helminthosporium sativum* Antifungal compounds enniatins A, A1, B, B1 have been reported to exibit antifungal activity against three strains of *Fusarium verticilloides*, one strain of *Fusarium*

sporotrichioides, *Fusarium oxysporum*, *Fusarium poae* and *F. tricinctum*, two strains of *F. proliferatum*, three strains of *Beauveria bassiana*, one strain of *Trichoderma harzianum*, two strains of *Aspergillus flavus,* one strain of *Aspergillus parasiticus*, *Aspergillus fumigatus* and *Aspergillus ochraceus,* and two strains of *Penicillium expansum*. (G. Meca et al., 2010).



Enniatin	R1	R2	R3
A	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$
A_1	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$	$CH(CH_3)_2$
B	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)_2$
B1	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$

Fig. 1 Antifungal Compounds

Pinus strobes have been reported to produce four compounds methyl (2Z,4E)-6(acetyloxy)-5-formyl-7-oxoocta-2,4-dienoate, 5-(hydroxymethyl)-2-(20,60,60-trimethyltetrahydro-2H-pyran-2- yl) phenol and (E)-5-(hydroxymethyl)-2-(60-methylhept-20-en-20-yl) phenol and these compounds have antifungal activity against both the rust fungus *M. violaceum* grown on MYP (malt, yeast, peptone media) Zhang et al., 2008 and *S. cerevisae*. (Mark W. Sumarah et al., 2011). In another studies a group of the four metabolites (Griseofulvin, Dechlorogriseofulvin, 8-dihydroramulosin, Mellein) reported to be isolatesd from *Nigrospora sp*. LLGLM003, reported fungi static activity in vitro against 8 plant pathogenic fungi: *B. cinerea, Colletotrichum orbiculare, Fusarium oxysporum f.sp. cucumerinum,*

Fusarium oxysporum f.sp. melonis, Pestalotiadiospyri, Pythiumultimum, Rhizoctonia solani and Sclerotinia sclerotiorum (Zhao et al., 2012).

Originally, the internal root bark of the plant *Ginkgo biloba* was the source of ginkgolides but later on a group of fungal isolates were screened and fermented on solid media and their metabolites were analyzed by TLC. The obtained potential ginkgolides producing fungus, in a study *Fusarium oxysporum*, was successively cultured in the liquid fermentation media. The ginkgolide B was successfully isolated from the metabolite and identified by HPLC/ ESI-MS and ^{13}C-NMR. The current research provides a new method to produce ginkgolide B by fungal fermentation, which could overcome the natural resource limitation of isolating from the leaves and barks of the plant *Ginkgo biloba* (Yuna Cui et al., 2012).

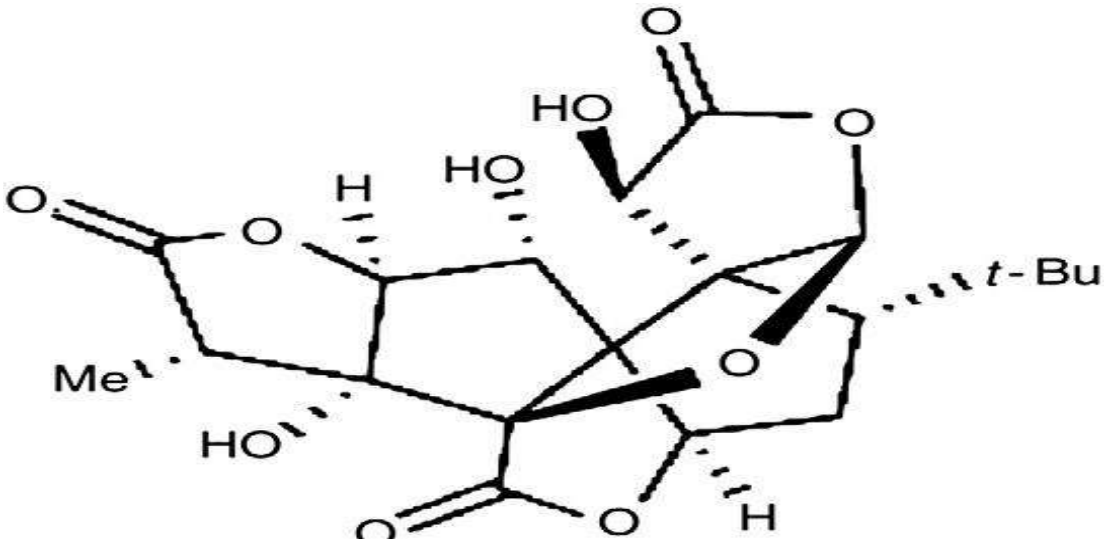


Fig. 2 Chemical structure of ginkgolide B

2.2.1 Antifungal activity of Alkaloid group

Indole derivatives of Alkaloid group, 6-isoprenylindole-3-corboxylic acid (2) showing Antifungal activity have been isolated from the endophyte *Colletotrichum sp*. showing growth inhibition property against phytopathogenic fungi; *Phytophthora capsici, Rhizoctonia cerealis,* and *Gaeumannomyces graminis var. tritici* (Lu et al., 2000). Another isolate of *Phomopsis sp.,* isolated from *Gossypium hirsutum* has yielded antifungual compounds, epoxycytochalasin H, cytochalasin N, and cytochalasin H in chloroform: methanol extract of it. And they were found active against Sclerotinia sclerotiorum, Bipolaris maydi, *Fusarium oxysporum*, Pyrocidine A and antimicrobial compound reported from Acremonium zeae, an endophytic fungus isolated from maize (Wicklow et al., 2005). Chaetomium globosum an endophytic fungus of Ginkgo biloba is reported to reservoir of antifungal metabolite gliotoxin (Li et al., 2011). Cryptocandin an antifungal compound was isolated from Cryptosporiopsis cf. quercina an endophytic fungus of Tripterigeum wilfordii and this was found active against plant pathogenic fungi Sclerotinia sclerotiorum and Botrytis cinerea (Strobel et al., 1999). graminis var. tritici and *Rhizoctonia cerealis*. 1-N-methyl albonoursin, an antibiotic alkaloid, was reported from endophyte isolated as a Streptomyces from perennial ryegrass and having morphological similarity to Acremonium (Gurney and Mantle, 1993). Antifungal mycotoxin fumigaclavine C and fumitremorgin C were isolated from Aspergillus fumigatus, an endophytic fungus of Cynodon dactylon (Cole et al., 1977).

2.3 **Antifungal activity of Terpenoids and steroids**

Anumber oof antifungal compounds have been reported to be of Terpenoids and steroids in nature, some impotents are inlisted in Table 2.

Table 2 Metabolites of endophytic fungi having antifungal activity—Terpenoids and steroids Groups

Sl. No	Host plant	Endophytic fungi	Biological activity	Functional metabolite reported	References
1	Phleum pretense	Epichloe typhina	Antifungal	Sesquiterpenes- Chokols A-G	Koshino et al., 1989b
2	Juniperus communis	Hormonemasp.	Antifungal	Enfumafungin	Pelaez et al., 2000
3	Artimisiaannua	Colletotrichum sp.	Antifungal (Phytophthora capsici, Helminthosporium sativum, Rhizoctonia cerealisand Gaeumannomyces graminis var. tritici	3b-hydroxyergosta-5-ene (29); 3-oxoergosta-4, 6,8 (14), 22-tetraene (30); 3b, 5adihydroxy-6b-acetoxyergosta-7, 22-diene (31) and 3b, 5adihydroxy-6b-phenylacetoxyergosta-7, 22-diene (32)	Lu et al., 2000
4	Mallushalliana	Alternaria brassicicola	Antifungal	Cerevisterol	Guet al., 2009
5	Cassia spectabilis (Leguminosae)	Phomopsis cassiae	Antifungal	Cadinanesesquiterpenes	Silva et al., 2006
6	Piper aduncum	Xylariasp.	Antifungal	Phomenone (27) Phaseolinone (28)	Silva et al., 2010
7	Arisaema erubescens	Phoma sp.	Antibacterial and Antifungal	b-sitosterol (33)	Wang et al., 2012

2. 3. Cytotoxicity activity:

Endophytic fungus Chaetomium sp. isolated from Salvia officinalis produced bioactive secondry metabolites which were rported to display antiproliferative activity against L5178Y mouse lymphoma cell line using the microculture tetrazolium (MTT) assay (Abdessamad Debbab, 2009. In another study, Bioactive Metabolites from the Endophytic Fungus *Stemphylium globuliferum* displayed a strong kinase inhibitor properties against Aurora-B, FLT3 kinases and CDK4/CycD1 which were the most susceptible to this natural bioactive metabolite (Abdessamad Debbab et al., 2009)

2.4 Hydrolytic enzymes and quorum sensing inhibitors:

There are a number of endophytic fungal species studied till date like; *Haplosporangium sp. Fusarium sporotrichioide, Cercospora sp., Papulospora sp., Phomopsis sp. Lasidiplodia sp., Papulospora sp. Fusarium graminearum Scopulariopsis costantini, Scopulariopsis sp. Trichoderma sp. and Fusarium semitectum* have been reported to demonstrate pectinase activity xylanase activity, amylolytic activity, Cellulase activity and Protease activity, (Rajesh et al., 2013).

2.5 Antithrombotic Activity

Some *Fusarium sp* CPCC 480097 *has* been reported to produce antithrombotic metabolites. The fibrinolytic activity of the purified antithrombotic agent from *Fusarium sp* was estimated by a modified fibrin plate method (Bin Wu, 2009).

2.6 Antiviral compounds

There are limited reports for number of compounds working as antiviral agents from endophytes. However, the fewer reported compounds that have already been isolated have been identified to show promising results and thereby provide an alternative means of antiviral drug production. The main limitation to antiviral compound discovery is most probably related to the absence of antiviral screening system in most of the compound discovery programs. In a report two novel compounds cytonic acid A ($C_{32}H_{36}$ O_{10}) and B ($C_{32}H_{36}O_{10}$) have been isolated from *Cytonaema sp*. These compounds are reported to work as novel protease inhibitors for human cytomegalovirus. Li et al., (2008 a, b) isolated an important antiviral compound Pestalotheol- C from the fungal endophytes Pestalotiopsistheae

of an unidentified tree on Jianfeng Mountain, China. The isolated compound expressed anti-HIV properties (Sanjana Kaul, 2012).

2.7 Antioxidant activity

Antioxidant activity from endophytic fungi from *Nerium oleander* has been reported by Wu-Yang Huang et al., 2007. Method for antioxidant activity was developed by Noro et al., 1983 with some modification (Kweon et al., 2001) and this protocol was used to measure xanthine oxidase activity with xanthine as the substrate The fungal strain *Chaetomium sp. NoS3* and the host plant *Nerium oleander* were both investigated for xanthine oxidase inhibition with allopurinol, rutin, and chlorogenic acid co-tested as positive controls. Xanthine oxidase inhibitory activity was expressed as the percentage of xanthine oxidase inhibition in the above assay system, calculated as (1 – B/A) X 100, where A and B are the activity of the enzyme without and with the test material, respectively.

2.8 Antitubercular compounds

The World Health Organization (WHO) ereported that currently 50 million people are infected and 1500 people die each hour from tuberculosis worldwide. After emergence and spread of Mycobacterium tuberculosis resistant strains to multiple drugs, the search for new anti mycobacterial agents is very necessary. The information based on medicinal plants as repository for fungal endophytes with metabolites containing biologically active compounds against various human pathogenic diseases for potential use in modern medicine have turned the attention of researcher to focus on endophytes . Endophytic fungi from *Garcinia sp.* are good source for exploring the possibility of new anti mycobacterial drugs. Phomoxanthone A and B isolated from the endophytic fungus Phomopsis sp. from Garcinia sp. in Thailand exhibits significant activity against M. tuberculosis (Isaka et al., 2001). *Phomopsis sp*.

isolated from *Garcinia dulcis* produced new metabolites as Phomoenamide and Phomonitroester inhibiting *M. tuberculosis* (Rukachaisirikul et al., 2008). Tenuazonic acid ($C_{10}H_{15}NO_3$) was isolated by bioassay guided fractionation of dichloromethane extract of *Alternaria alternata*. It was found to be active against Mycobacteriumtuberculosis at MIC of 250 µg/ml concentrations (Sonaimuthu et al., 2011). Diaporthesp, isolated from*Pandanus amaryllifolius leaves* produced two new benzopyranones diaportheone A and B. Both compounds inhibited the growth of virulent strains of *Mycobacterium tuberculosis* (Bungihan et al., 2011).

2.9 Isocoumarins and chromones

Many of the bioactive metabolites have been isolated from various endophytic fungi and some of them are briefly detailed in this section. Antifungal metabolite mullein derived from Pezicula sp. is a member of isocoumarins group of natural products. It is detailed to be strongly fungicidal, herbicidal and algicidal in its chemical proprties (Schulz et al., 1995). Another Penicillium sp. an endophytic fungus of Alibertia macrophylla (Oliveira et al., 2011) has been reported to produce a potential bioactive metabolite; 7 hydroxymellein, which has strong antifungal activity against *Cladosporium cladosporioides* and *C. sphaerospermum*.

2.10. Other Bioactive metabolites

In literature a number of compounds have been reported showing strong antimicrobial activity isolated from endophytic fungi having antifungal activity. One of them areJesterone and hydroxyjesterone and these bioactive antifungal compounds were isolated from Pestalotiopsis jesteri an endophytic fungus of Fragraea bodenii (Li and Strobel, 2001). In a separate group of study on bioactive metabolites from endophytic fungi, Liu et al., 2009 have reported pestalofones groups of bioactive compounds. These coumpounds were proved

potent antifungal agents. Similarly Brefeldin A belonging to lactone group has antifungal and antimicrobial properties has been reported from several endophytic fungi belongings to extended range of host plants, Aspergillus clavatus and Paecilomyces sp. inhabilte inside Taxus mairei. In *Quercus variabilis*, *Torreya grandis* and *Cladosporium sp.* were observed to be a major inhabitating fugal species (Wang et al., 2002, 2007). *Pezicula sp*. has been reported to produce fungicidal molecule (Schulz et al., 1995). Trichoderma citrinoviride, an endophytic fungus of cork oak, reprted to produce peptaibols, a peptide mixture, it displayed strong antifungal activity against fungal pathogens; *Candida albicans, Sclerotium rolfsii, Sclerotinia, Phoma exigua, scleratiourm, Fusarium sp.* are involved in oak decline (Maddau et al., 2009). Because of above literature discussion it is clear fact that fungi are expanding their boundary of niche by colonizing the living plant as well, while they are saprophytic and were essentially living on dead organic matter. *Trichoderma sp.* and *Beaveria bassiana*, economically very important, have been two such species to be isolated from living healthy plants as endophyte with higher frequency.

2.11 Biotechnological Applications of Endophytes

Endophytes have high ability to produce various novel and known enzymes which could be used in various biotechnological applications like environmental applications of degradation enzymes, medical applications, and biotransformation of organic compounds with many advantage over other methods (Firáková et al., 2007, Pimentel et al., 2011, Sury et al., 2012).