Endophytes: A hidden treasure inside plant

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Abstract
Nowadays, bioactive compounds gaining importance in pharmaceutical and agriculture industry due to their multitasking role in human and plant health. Endophytic microorganisms have a unique property to synthesize bioactive compounds having negative effect on plant and human pathogens. In the last few years, many bioactive compounds with insecticidal, antimicrobial and anticancer properties are discovered from endophytes by eminent scientist of the world. Bioactive compounds produced by endophytes alone or in conjunction with host plant have ecofriendly nature, toxic for pathogens and harmless for plant, animals and humans. Bioactive compounds of endophytes are secondary metabolite of valuable biotechnological application, inoculation of these compounds or sole microbial inoculation improves plant health. Enzymes produced by endophytes help plant to develop systematic resistance against invading pathogens. Endophyte inoculation in plant triggers production of plant growth hormone which play essential role in plant growth and development. Endophytes also play an important role in abiotic stress management and helpful for plant to withstand during drought and salt stress conditions. The high diversity and their adaptation to various stress condition, untapped sources of bioactive compounds, effectiveness and ecofriendly nature makes it suitable candidate to use in sustainable agriculture production and in pharmaceutical industries. The present review emphasize on bioactive compounds of endophytes and their role in agriculture and pharmaceutical industries.

Keywords: antimicrobial, bioactive compounds, endophytes, phytohormones

Introduction
Endophytes are symbiotic microorganism lives inside the different tissue part of plant like nodule, root, bark, leaves and stems without causing any damage to plant or plant part. Approximately, 300000 higher plants present all over the world and every plant are accompanied with one or more endophytic microbes. Recent studies have revealed that endophytes associated with plant in a variety of relationship like mutualistic, commensalism, cooperation, pathogenic. In turn plant provides nutrient and shelter to the endophytic fungi. During mutualistic or symbiotic life cycle in plant endophytic fungi protect plant from drought, increased resistance to disease, protect plant from nematode attack and grazing, provide resistance to heat, and help in plant growth promotion. All of the symbiotic relationship of endophytes with plant is accompanied through production or secretion of bioactive compound. A chemical compound of biological origin possesses antimicrobial activity against other microorganisms of same and different species can be defined as low molecular weight compounds or bioactive compounds. These bioactive compounds are of different nature like terpenoids, steroids, xanthenes, phenols, isocoumarins, perylene derivatives, quinines, furandiones, terpenoids, depsipeptides and cytochalasins. Endophytes have a resistance mechanism to overcome pathogenic invaders by producing secondary metabolites bearing antimicrobial activity. More than 8600 bioactive compounds have been reported from endophytes showed antimicrobial, anticancer, antiviral and antiadiebatic properties. New organisms and many novel natural products from endophytic microbe can inhibit or kill a wide variety of harmful microorganisms like bacteria, fungi, viruses and protozoans that affect humans and animals. Endophytes do produce secondary metabolites in culture, but the temperature, composition of the medium and the degree of aeration will affect the amount and kind of compounds produced. Endophytic microorganisms upon met abolomic investigations revealed that they are potential, reliable and promising source of novel organic natural metabolites exhibiting a wide range of promising bioactive properties viz. antioxidant, antimicrobial, enzyme inhibitive, antiparasitic, cytotoxic, immunosuppressive, neuroprotective, insulin mimetic and many more.
Due to the extreme medical importance of these metabolites, there is an increasing interest in studying fungal endophytes as alternative potential source of such useful functional metabolites. Therefore, keen studies on endophytes are required for the good and economical production of these metabolites from endophytic microbes as their production from host plant is comparatively costlier and time consuming. A wide range of endophytic applications have extensively been discussed by many researchers.

**Fig 1: Use of various bioactive compounds of endophytes in different industries**

So far, studies reported a large number of anti-microbial compounds isolated from endophytes, belonging to several structural classes like alkaloids, peptides, steroids, terpenoids, phenols, quinines and flavonoids. The three isolated compounds, melleolides K, L and M. from *Armillaria mellea* which showed anti-microbial activity against gram positive bacteria, yeast and fungi. Previously these antibacterial sesquiterpenoids, melleolides B-D, were yielded from *Armillaria mellea* were isolated by Arnone. A compound polyketide citrinin produced by endophytic fungus *Penicillium janthinellum* from fruits of Melia azedarach, presented 100% antibacterial activity against *Leishmania sp*. Podophyllotoxin (A non alkaloid lignin) and its analogues are clinically relevant mainly due to their antiviral and anticancer activities, further they are the precursors of many other useful anticancer drugs including etoposide and teniposide (Aly et al. 2013). Various novel microbial sources for podophyllotoxin include *Aspergillus fumigatus* isolated from *Juniperus communis*. Hence there is a need for naturally occurring compounds which are less toxic, target specific, easily degradable and can compete with synthetic pesticides. It is believed that screening of antimicrobial compound from endophytes is a promising way to overcome the increasing threat of drug resistant microbes of human and plant pathogen. Here, we are covering the metabolites obtained from various endophytic microbes, belonging to various classes along with unidentified fungi and their potential as antifungal agent. Many of these compounds are shown in Table 1.

**Distribution and diversity of endophytes**

Almost all vascular plant species examined to date were found to harbor endophytic bacteria or fungi. Surveys of various hosts during the past 20 years have demonstrated that the colonization of land plants by endophytes is ubiquitous. Endophytes are detected in plants growing in tropical, temperate and boreal forests with the hosts ranging from herbaceous plants in various habitats including extreme arctic, alpine and xeric environments to mesic, temperate and tropical forests. Moreover, the colonization of endophytes in marine algae, mosses, ferns, fern allies, numerous angiosperms, gymnosperms including tropical palms, broad leaved trees, estuarine plants, diverse herbaceous annuals, many deciduous and evergreen perennials has also been recorded. Endophytes are found in a wide variety of plant tissue types, such as seeds, ovules, fruits, stems, roots, leaves, tubers, buds, xylem, rachis and bark (Kaul et al. 2013). A perusal of the literature over the past decades indicated many ethnomedicinal plant species with rich botanical history, sampled from unique ecologica niches and species are known to harbor potential endophytic microbes (Strobel and Daisy, 2003). There are approximately 300,000 different plant species on our planet. An estimate of 1 million endophytes seems reasonable if each individual higher plant hosts an average of one to few endophytic strains. As a matter of fact endophytes are important components of microbial biodiversity. Commonly, several to hundreds of endophytic species can be isolated from a single planta nd among them, at least one species showing host specificity. The environmental conditions under which the host is growing also effect the endophyte population and the endophyte profile may be more diversified in the tropical area. Endophytes are presumably ubiquitous in the plant kingdom with the population being dependent on host species and location. Many studies have tried to determine whether endophytes exhibit tissue-specificity or host-specificity but the results are contradictory with indications of both host-specificity and host generalism.

**Functions of endophytes**

Some endophytes have no apparent effects on plant development and growth, live on compounds produced by plant, they are known as commensal endophytes. Some endophyte have beneficial effects on host plant such as production of plant growth hormones, protection against biotic stress by targeting invading pathogens, production of antibiotic against invading pathogens and induced systematic resistance inside host plant (Hardoim et al 2015). A third group of endophytes showed detrimental effect on plant. Generally, endophytes have neutral effect on plant during normal growth conditions while they are more beneficial and showed positive effect on plant during intensive condition during plant life cycle, in short during intensive condition endophytes acts like an army provide defence as well as need based requirement to the host plant (Verma et al 2009). E.g. fungus *Fusarium verticillioides* play both role as a pathogen as well as beneficial endophytes in maize. Same endophyte acts differently on different plant, for one plant acts in symbiotic manner for other plant acts in detrimental manner. Even for the same plant endophytes have different role, in one phase of life cycle become detrimental and in other stage of life cycle and harsh environmental condition become beneficial. The balance between two states is depends on type of species, but also depends on environmental conditions (abiotic stress). The behavior of endophytes inside plant host varies in different stages of life cycle. E.g. *F. verticillioides* repress growth of additional pathogenic fungus, *Ustagiomaydis*, protect their host from diseases. Clavicipitaceous fungi produced neurotoxic indole.
diterpenoid alkaloids in infected grass which leads intoxication in grazing cattles (Aly et al 2011) [2]. Some other compounds produce by endophytic fungi are provide defense against insect herbivores (Verma et al 2009) [33]. Moreover, some studies suggested difference present on some part of antibacterial, antiviral antifungal and insecticidal compounds from fungal endophytes and some of these endophytes are pass on horizontally, and form like local infections within their hosts. Not all horizontally transfer fungal endophytes produced beneficial compounds, and suitable to the repeatedly small window having chances for ecology as well as functioning of positive endophytes (Higginbotham et al 2013) [10]. Various microbes especially bacteria and fungi, colonize inside the internal structure of specific host plant. Inside the host paint endophytic microbes (both bacteria and fungus) interact with host plant as well as some nearby microbes. Endophytic arbuscular mycorrhizal fungi (AMF) form specialized structures called arbuscules where exchange of nutrients between fungus and host plant takes place. Plant derived carbon source exchanged with AMF derived essential nutrient of plant like phosphate (Pi), nitrogen (N) and potassium (K) elements. Plant derived cytoplasmic sucrose is transported to periarbuscular space where it is converted to hexose sugar (glucose). AMF derived important plant nutrient Pi and N are transported to fungal cytoplasm where it is converted to Pi and arginine. Phosphate (Pi) finally transported to host cytoplasm whereas arginine is initially converted to urea (Ur) and then to ammonium (NH₄). Some other important compounds of plant importance are also formed by AMF transported Nitrogen (N) like plant hormones i.e. auxins (IAA), cytokinins (CK), gibberellins (GA) and volatile organic compound having role in plant defense and development. Resistance in host plant is induced upon entry of endophytic microbe because various microbial structure such as flagella, microbe derived proteins, pili, lipopolysaccharides of cell membrane, secretion system of endophytic microbes, various effectors and antibiotic molecule, jasmonic acid, salicylic acid of endophytic microbe origin triggers induced systematic resistance.

**Fig 2:** Schematic representation of endophyte isolation, purification and function of endophytes in host plant

Endophytes play an important role in defense system of plant. As life cycle of endophytes shorter than life cycle of host plant, so they develop in very large number inside the host plant, resultant in higher collection of antagonistic form that resistance against plant pathogens and harbiovores. Communications and interactions among large endophytic population brought through signal exchange via growth factors and antibiotic molecule. Siderophore production by microbes causes sequestration of iron (Fe) molecule necessary for growth of plant pathogens. Therefore, this indirect mechanism of iron sequestration protects plant from pathogenic fungi attack (Hardoim et al 2015) [9] There is increasing evidences that at a suitable, interactions among valuable microbes and plants cause an immune response in plants similar to that of pathogens but later on, mutualists run away host defense responses as well they are able to successfully colonize plants (Aly et al 2011) [2]

**Anti-microbial effect of endophytes**

Bioactive metabolites are those low molecular weight organic molecule which are produced by microorganisms that are active at low concentration against other microorganisms (Guo et al. 2008) [18]. Endophytic microbes produced secondary metabolites active against pathogenic microbes (Tan and Zou, 2001). In the previous studies, it was reported that a large number of bioactive compounds isolated from endophytes, belonging to different classes like peptides, steroids, terpenoids, phenols, alkaloids, quinines and flavones (Yu et al., 2010) [36]. Extraction of novel antibiotic metabolites from endophytes is an alternative reduced the increasing level of drug resistance in pathogens. Till date a fewer effective antibiotic are discovered against a wide range of pathogens, less target specificity probably leads to unfavorable return on investment (Yu et al., 2010; Song, 2008) [36, 29]. The antimicrobial compounds are not only used
as drugs for human kind but can also used to control food borne diseases and food spoilage, a serious issue nowadays (Liu et al., 2008)\textsuperscript{[18]}. Many bioactive compounds are isolated from different endophytic microbe present on different host shows antifungal activities e.g. Xyleria sp. Isolated from different host such as sordaricin shows antifungal activity against Candida albicans. Similarly, bioactive metabolite mellisol, 1, 8-dihydroxyanaphth 1-0-a-glucopyranoside had activity against herpes simplex virus (Pittayakhajonwut et al., 2005)\textsuperscript{[22]}. The bioactive compounds isolated from endophytic fungus Xyleria sp YX-28 isolated from Ginkgo biloba L. plant was identified as 7-amino-4-methylcomurin. This compounds had a broad spectrum inhibitory activity against several food borne and food spoilage microorganisms including E. coli, S. typhimurium, S. aureus, Yersinia sp., Shigella sp, V. hameolyticus, V. parahemolyticus, C.albicans and A.niger (Liu et al., 2008)\textsuperscript{[18]}. Another strain of endophytic fungus Xyleria sp. Isolated from Abiescholophylla was characterized as a producer of griseofulvin, antifungal antibiotic use gainst treatment of human and animal skin diseases (Park et al., 2005)\textsuperscript{[21]}. Further studies revealed high antifungal activity of endophytic fungus Xyleria sp. producing griseofulvin against plant pathogenic fungi also. Another fungus C. globosum isolated from Ginkgo biloba producing Chaetomugnilin A and D had antifungal activity (Qin et al., 2009)\textsuperscript{[24]}. Compounds Cytosporone B and C were isolated from Phompsis sp. Endophytic fungus grown inside mangrove plant tissue showed antifungal activity against C.albicans and F. oxysporum with MIC value ranging from 32 to 64 mg MI\textsuperscript{[Huang et al., 2008]}\textsuperscript{[11]}. Endophytic fungus present in inner tissue of Quercus variabilis possessed growth inhibition against a wide range of fungi and bacteria. A compound kown as brefledin A, a lactone produced from endophytic fungus Cladosporium sp. Shows most promising antifungal activity against different plant pathogenic fungus (Wang et al., 2007)\textsuperscript{[34]}. An endophytic Streptomyces sp., from a fern-leaved grevillea (Grevilleapeteridifolia) in Australia was described as a promising producer of novel antibiotics, kakadumycin A and echinomycin. Kakadumycin A is structurally related to echinomycin, a quinoxaline antibiotic and presents better bioactivity than echinomycin especially against Gram positive bacteria and impressive activity against the malarial parasite Plasmodium falciparum (Castillo et al., 2003)\textsuperscript{[5]}. An endophytic fungus Streptomyces sp. produced a peptide antibiotic Coronacyn which is active against pythioceous fungi, the human fungal pathogen Cryptococcus neoformans and the malarial parasite Plasmodium falciparum.

B. pumilus isolated from cassava cultivated by Amzon Indian tribes produced a lipopeptide named pumilacidin have antifungal activities. Two new bioactive metabolites, ethyl 2, 4-dihydroxy-5, 6-dimethylbenzoate and phomopsilactone were extracted from an endophytic fungus Phomopsiassae isolated from Cassia spectabilis and had strong antifungal activity against two phytopathogenic fungi, Cladosporium cladosporioides and C. sphaerospermum (Silva et al., 2005)\textsuperscript{[28]}. The polyketidecirtrin produced by endophytic fungus Penicillium janthinellum from fruits of Melia azedarach, presented 100% antibacterial activity.

### Table 1: List of bioactive compounds and their function

<table>
<thead>
<tr>
<th>Endophytes</th>
<th>Host plant</th>
<th>Compound</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pestalotiopsis microspora</td>
<td>Taxus taxifolia</td>
<td>Torreyanic acid</td>
<td>antimicrobial</td>
<td>Lee et al. (1996)\textsuperscript{[16]}</td>
</tr>
<tr>
<td>Trichoderma harzianum</td>
<td>Ilex cornuta</td>
<td>Trichodermin</td>
<td>antimicrobial</td>
<td>Chen et al. (2007)\textsuperscript{[6]}</td>
</tr>
<tr>
<td>Chloridium sp.</td>
<td>Azadirachta indica A Juss</td>
<td>Javanicin</td>
<td>antimicrobial activity against P. aeruginosa and P. fluorescens</td>
<td>Kharwar et al. 2009</td>
</tr>
<tr>
<td>Phomopsis sp</td>
<td>Erythrina cristagalli</td>
<td>Isolavonoids</td>
<td>Antimicrobial</td>
<td>Red et al 2011</td>
</tr>
<tr>
<td>Emericilla sp</td>
<td>Aegiceras</td>
<td>Aegiceras cornucaulatum</td>
<td>Antiviral</td>
<td>Zhang et al 2011 \textsuperscript{[17]}</td>
</tr>
<tr>
<td>Eupenicillium parvum</td>
<td>Azadirachta indica</td>
<td>azadirachtin A and B</td>
<td>natural insecticides</td>
<td>Kusari et al. 2011 \textsuperscript{[11]}</td>
</tr>
<tr>
<td>Chaetonium chiversii C5-36-62</td>
<td>Ephedra fasciculata</td>
<td>Radicicol</td>
<td>Cytoxic</td>
<td>Turbyville et al 2006 \textsuperscript{[32]}</td>
</tr>
<tr>
<td>Phomopsis sp</td>
<td>Plumeria acutifolia</td>
<td>Terpenoids</td>
<td>Antiviral</td>
<td>Kusari et al 2011 \textsuperscript{[11]}</td>
</tr>
<tr>
<td>Massodor albus</td>
<td>Cinnamomum zeylanicum</td>
<td>1-butanol, 3-methyl-acetate</td>
<td>Antimicrobial</td>
<td>Strobel &amp; Daisy 2003 \textsuperscript{[80]}</td>
</tr>
<tr>
<td>Nigrospora sp. YB-141</td>
<td>Azadirachta indica</td>
<td>Solanapyrone Nigrosporalactone Phomalactone</td>
<td>Antimicrobial</td>
<td>Xu et al 2007</td>
</tr>
<tr>
<td>Xyleria sp. YX</td>
<td>Juniperus communis L</td>
<td>Enfumafungin</td>
<td>Antifungal activity against C. albicans</td>
<td>Aly et al. 2011 \textsuperscript{[2]}</td>
</tr>
<tr>
<td>Eupenicillium sp.</td>
<td>Xanthium sibiricum</td>
<td>Eupenicinocids A and B</td>
<td>Antibacterial</td>
<td>Li et al. 2014</td>
</tr>
<tr>
<td>Gastrodia Elata</td>
<td>Armillaria amellea</td>
<td>Sesquiterpene aryl esters</td>
<td>Antimicrobial activity</td>
<td></td>
</tr>
<tr>
<td>B. pumilus MAIIIM4a</td>
<td>cassava</td>
<td>Pumilacidin</td>
<td>inhibitory activity against the fungi</td>
<td>Melo et al. 2009</td>
</tr>
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</table>

\textsuperscript{“1663”}
### Conclusion

Endophytes are a poorly investigated group of microorganisms capable of synthesizing bioactive compounds that can be used to combat numerous pathogens. These have been dependable sources of bioactive and chemically novel compounds and have proven to be useful for novel drug discovery. Biotransformation, methods have a wide range of uses, particularly in the production of numerous bioactive compounds, as antimicrobial (vanillin, essential oils), antifungal and antiviral (alkaloids), antioxidant (eugenol), antiinflammatory (cineole) etc. Moreover, endophytes are good candidates for plant growth promotion in various crops through their various effects on plants like phytohormone production, nutrient absorption, and tolerance to biotic and abiotic stress. It is imperative to review and highlight the previous successes, on-going research and latest developments in research associated with endophytic microorganisms to draw the attention of the research community toward this emerging field and possible exploitation of the available sources for their therapeutic uses in various fields, such as the medical, pharmaceutical, food and cosmetics.

### References


