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The fungal endophytes: Sources and future prospects

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Abstract
The Plants, animals and microorganisms have provided a large variety of biologically active compounds which have wide applications in human health and diseases. Many diseases such as HIV, cancer, tuberculosis are emerging in society with new mode of actions creating a need for the discovery or development of novel products for human health and welfare. However, the problem is the emerging antibiotic resistant varieties of the pathogens casing such deadly diseases. To overcome this problem researchers have shown new untapped area where fungal endophytes can be exploited. These fungal endophytes have come at glance due to broad range of hosts in biodiversity. A large number of endophytic fungi produce valuable Bioactive compounds of medicinal, industrial and agricultural importance. Cryptosporiopsis, Taxomyces, Colletotrichum, Pestalotiopsis, Muscodor, Alternaria are some of the known examples of fungal endophytes in nature. The present review explores various sources and future prospects of endophytic fungi.

Keywords: Antibiotic resistance, Endophytic fungi, biodiversity sustainability

1. Introduction
The World Health Organization (WHO) estimates that in low and middle income countries 30% of the total 70 million annual deaths occur due to increased multidrug resistant ability of microbes [44]. Multidrug resistance occurs when pathogens undergo spontaneous or induced mutation [28, 18, 30, 35]. Hence these conditions demand immediate action to understand clearly the epidemiology of the resistant pathogens, the mechanism of resistance and the treatment available. To overcome this problem, we can use and aim for purified bioactive metabolites from plant and microbes. As far as microbial world concerning bioactive metabolites, a large number of microbial domains have shown to have potential clinical applications. One of them being fungal domain [18, 38, 17, 19, 16, 20].
Back in 1928, Alexander Flemming discovered antibiotic penicillin from *Penicillium notatum* which was later used during World War II [28, 18]. Afterwards in 20th century, there was the explosion of new antibiotics such as chloramphenicol, streptomycin, tetracycline, griseofulvin, cyclosporine, taxol from different fungal species therefore, this period is known as “antibiotics era” [28, 18, 30, 35]. In 21st century, novel antibiotic compounds were isolated from different fungal species due to their ability to produce potential pharmaceutical products. However, there exists a growing demand for the discovery of novel antibiotics, chemotherapeutic agents and agrochemicals from fungal species [30, 41]. Hence forth, the persistent efforts and search by researchers lead to the discovery of a whole new microbial society namely "Endophytes" [41, 18, 38, 42]. Evolutionary proof in the form of the Fossils shows that fungal symbionts have been associated with plants from the Ordovician period of approximately 400 million years ago, when plants first became established on land [22] migrating from aquatic to terrestrial habitats (Yang *et al*., 1999). De Barry (1866) coined the term 'endophyte' to detect fungi which reside inside the healthy tissues of the host plant far from epiphytes (De Barry et al. 1866). After 100 years, Carroll (1986) used term 'endophyte' for those organisms that causes asymptomatic infections within the plants. Petrini (1991), elucidated Carroll's definition as a commensalism in plants. Wilson (1995), further expanded endophytes and included both fungi and bacteria as a commensal [30, 35, 35]. At glance endophytes are defined as organisms that inhabit plant tissues at some stage in their life cycle without causing apparent harm to their host [24, 38, 42]. Endophytes lives, reproduce and forms mycelia that grow between the cells of a plant, more in the leaf sheaths and reproductive structures [38, 42, 35].
Endophyte are symbiotically associated with the seeds even during seed germination and get

transferred to the plant during its growth and development. However, endophytes can turn off this symbiotic lifestyles and mutualistic benefits due to the influence of host genotypes. Further, the host range of endophytes is inadequately defined and includes both monocot and dicot species. Endophyte host plant describe adaptive symbiosis. Some endophytes have evolved with a high degree of suppleness to enter between genetically distinct plant species to provide endophytes an option to develop habitat range. Endophytic microbes can have intense effects on plant ecology, their fitness and are able to produce large number of bioactive agents [38, 42, 35]. The present review article is about sources of Endophytic fungi and their future prospects.

2. Endophytes versus Epiphytes

On Contrary to epiphytic fungi, the fungal species which grow inside plant tissues are endophytes. The two communities i.e. epi and endo-phytes have rarely been compared [6]. In practice to study endophytes verses epiphytes, surface disinfection of plant surfaces is a key point and using distilled water, 4% sodium hypochlorite and 70% ethanol is recommended. Temporally as well as practically, the distinction between endophytes and epiphytes is often arbitrary. Many horizontally transmitted endophytes presumably start growing on the surface of the leaf before penetration. Also, endophytes may become epiphytes when internal tissues are exposed, and may protect the exposed tissues from the environment [6, 42, 32]. Thus, an epiphyte that survives in culture might be assumed to be an endophyte [6]. Hence, at glance, the distinction between endophytes and epiphytes needs further exploration.

3. Biodiversity of fungal endophytes in India

India has about 329 million hectares geographical area of which a coastline of over 7500 km. The ecosystem diversity of the India is enormous, ranging from sea level to the highest mountainous ranges in the world; hot and arid conditions in the northwest to cold arid conditions in the trans-Himalayan region; tropical wet evergreen forests in Northeast India and the Western Ghats; mangroves of Sundarbans and fresh water aquatic to marine ecosystems [35, 22,]. Although many studies illustrate endophyte diversity in different ecologies, there is no reliable estimation of the number of endophytic species, of their host- and tissue-selectivity, since environmental factors have a complex effect on these features. Endophytes are reported from plants found in various environment including tropic, temperate, aquatic, oceans, xerophytic, deserts, Antarctic, geothermal soils, rainforests, mangrove swamps and also coastal forests (Rodriguez *et al.*, 2008; Strobel *et al.*, 2002; Suryanarayanan *et al.*, 2002; Yang *et al*., 1999; Merlin, 1922). Plants have ethnobotanical history that are related to specific applications for selected study. As far as India is considered Karnataka, Tamil Nadu, Bihar and Uttar Pradesh have great biodiversity for endophytic fungi (Suryanarayanan *et al.*, 2002; Tiwari 2015).

Table 1: Distribution of plants under different forms/habits

Sr. No	Habit	Number of plant species
1	Herbs	191
2	Trees	122
3	Shrubs	118
4	Climbers	69
5	Total	500

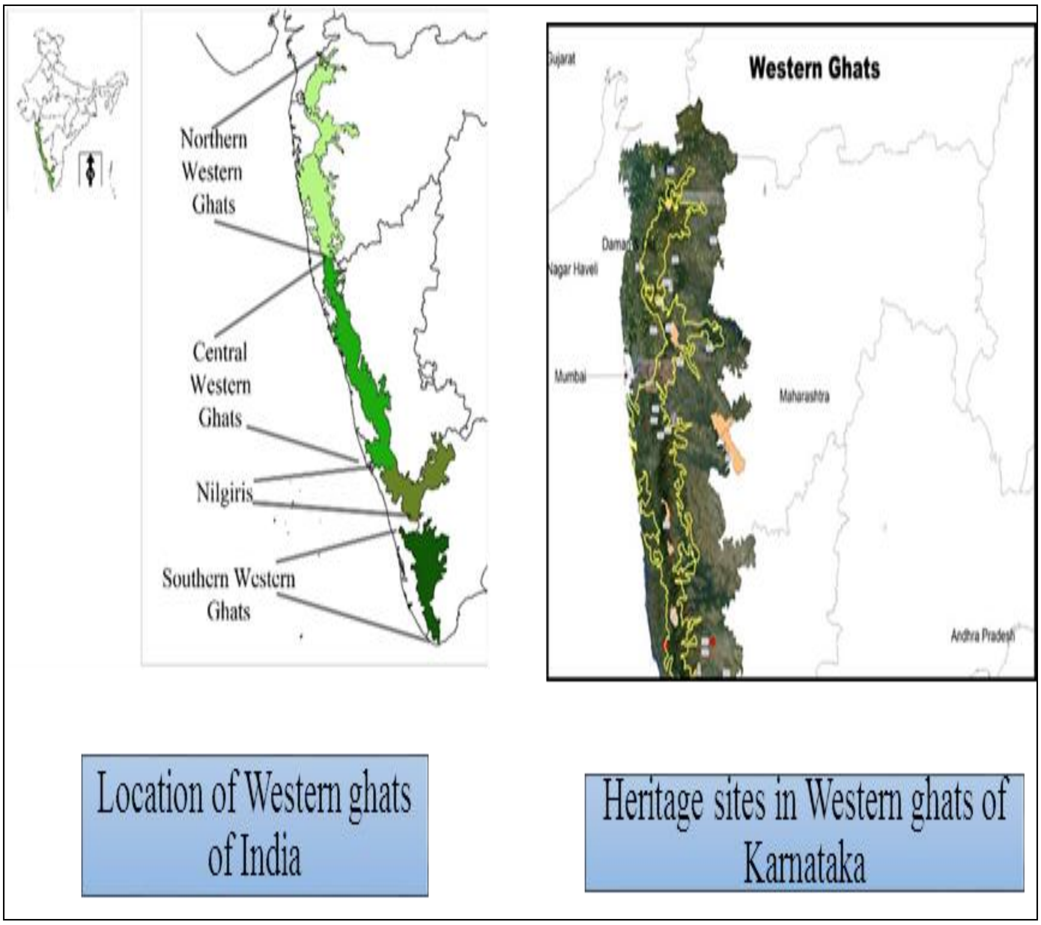

Fig 1: Location and heritage sites of Western Ghats of India

Fig 2: some valuable medicinal plants from Indian Biodiversity

Table 2: Endemic plant species in Western Ghats of India

S. No.	Karnataka	Kerala	Maharashtra
1	*Aglaia elaeagnoidea*	*Artocarpus hirsutus Lam.*	*Artocarpus hirsutus Lam.*
2	*Artocarpus hirsutus Lam*	*Cinnamomum wightii Meisner*	*Ervatamia heyneana Cooke.*
3	*Cayratia pedata*	*Diopyros paniculata Dalz.*	*Rauvolfia serpentina (L.) Benth.*
4	*Diopyros paniculata Dalz*	*Rhododendron arboreum*	-

4. Classification of Endophytic fungi

There are two major groups of endophytic fungi have been reported based on evolutionary relatedness, taxonomy, plant hosts, and ecological functions (Table: I). The *Clavicipitaceous* endophytes (C-endophytes), which infect some grasses; and the *nonclavicipitaceous* endophytes (NC-endophytes), which can be recovered from asymptomatic tissues of nonvascular plants such as ferns and allies, conifers, and angiosperms (Jesus *et al.*, 2014; Rodriguez *et al*., 2008; Bacon *et al.*, 1977).

Apart from these two main groups there are four classes of endophytic fungi

I) Class I Endophytic fungi
II) Class II endophytic fungi
III) Class III endophytic fungi
IV) Class IV endophytic fungi

These four classes are explained as follows.

The Class I Endophytic fungi are Clavicipitaceous endophytes of grasses which were first reported by European investigators in the late 19th century from seeds of *Lolium temulentum*, *Lolium arvense*, *Lolium linicolum*, and *Lolium remotum* (Rodriguez *et al*. 2008). From their earliest discovery, investigators hypothesized a link to toxic syndromes experienced by animals that consume infected tissues. However, these hypothesis were largely untested until Bacon *et al.* (1977) linked the endophyte Neotyphodium coenophialum to the widespread occurrence of summer syndrome toxicosis in cattle grazing tall fescue pastures (Festuca arundinaceae). As this hypothesis became widely known, investigations on endophyte natural history, evolution, ecology, and physiology followed [38].

The Class II endophytic fungi consists a diversity of species, prominently related to the Dikaryomycota (Ascomycota or Basidiomycota) although, mostly belonging to Ascomycota and Basidiomycota. The Basidiomycota includes Agaricomycotina and Pucciniomycotina. A *Phoma* spp. in *Calluna vulgaris* was first reported by Rayner in 1915 as an II class of endophytes. These species are always colonized in all the parts of the plant including the seed coat. According to recent analysis *Phoma* spp. are common root endophytes that confer fitness benefits to plants.

Table 3: Symbiotic criteria used for characterization of fungal endophyte classes

Characteristics	Class I	Class II	Class III	Class IV
HA & NHA	NHA	NHA & HA	NHA & HA	NHA & HA
In planta biodiversity	Low	High	High	Unknown
In planta colonization	Extensive	Extensive	Limited	Extensive
Tissues colonized	Shoot & rhizome	Shoot, root & rhizome	Shoot	Root
Host range	Narrow	Broad	Broad	Broad
Transmission	Vertical & Horizontal	Vertical & Horizontal	Horizontal	Horizontal
Growth	Above and below ground tissues	Above and below ground tissues	Above ground tissues	Below ground tissues

During the 20th century, brown alga *Ascophyllum nodosum* was reported which requires the fungus *Mycophycia ascophylli* for normal growth and development (Rodriguez *et al*., 2008).

The Class III endophytic fungi are differentiated on the basis of their occurrence i.e. above-ground tissues; the formation of highly localized infections; horizontal transmission; the potential to confer benefits on hosts that are not necessarily habitat-specific; and extremely high in planta biodiversity. These class includes the hyper diverse endophytic fungi associated with leaves of tropical trees [2, 15], as well as above-ground tissues of nonvascular plants, seedless vascular plants, conifers, and woody and herbaceous angiosperms in biomes ranging from tropical forests to boreal and Arctic/Antarctic communities [7, 29, 37].

These Class of endophytes are found in flowers and fruits, in asymptomatic wood and inner bark [40]. The Endolichenic fungi were also found under class III endophytes. [29] recovered more than 80 endophyte species from *Juniperus communis* in Switzerland [17] isolated 78 species from leaves and twigs of *Quercus petraea* in Austria. Like, Class II endophytes, majority of the Class III endophytes are members of the Dikaryomycota (Ascomycota or Basidiomycota), with inference to Ascomycota. Members of the Basidiomycota belonging to the Agaricomycotina, Pucciniomycotina and Ustilaginomycotina [37, 24].

The Class IV endophytes were revealed during the study of ectomycorrhizal fungi when [25] observed a brown to blackish, pigmented fungus associated with terrestrial plant roots that she called MRA. These species were often found with mycorrhizal fungi and referred to as *pseudomycorrhizal* fungi [25]. Presently, these fungi are referred to as dark septate endophytes (DSE) and are grouped together as Class IV endophytes. These Class IV endophytes are distinguished upon functional group, based on the presence of darkly melanised septa, and their restriction to plant roots. However, almost a century after their discovery, little is still known about the role of these mysterious and rather elusive fungal symbionts (Rodriguez *et al.*, 2008).

5. Sources for Endophytic fungi

5.1 Fungal endophytes from Grass

In India first, Sydow (1914) reported *Balansia andropogonis* Syd. & E.J. Butler from *Andropogon aciculatus* Retz. After 50 years in 1964 Govindu and Thirumalacher reported endophytes associated with grass. Total grass species 25-30% are endophytes which can play role in plant society's [8]. Based on the findings from microscopy examination C.R. Rodriguez *et al.*, 2007 explained the growth of Epichloë and Neotyphodium endophytes in host grasses.

According to Schulz (1998) [39] vascular bundles of Neotyphodium endophytes contain few hyphae in natural associations with grasses (Christensen *et al*., 2001). In 1997 Janardhanan and Ahmad reported *Balansia* and its anamorphic state *Ephelis* from various grasses from Karnataka, Bihar, Uttar Pradesh and Tamil Nadu [41].

5.2 Fungal Endophytes from medicinal plants

Now a days medicinal plants are under limelight due to their attention as they produce novel secondary metabolites against different pathogens. A number of medicinal plants have been studied for the isolation and their antibacterial or antifungal activity. Mainly *Trigonella foenum graecum (L), Silybum marianum (L), Allium sativum (L), Allium cepa (L), Mamordica chaeantia (L), Camellia sinensis (L), Azadarichata indica, Catharanthus roseus, Parthenium hysterophorus, Ficus religiosa, Coffea arabica (L). Nothapodytes nimmoniana, Pongamia, Ashwagandha, Taxus brevifolia. These plants* were also used for the study of fungal Endophytes by many researchers. Mane *et al*., 2017 isolated 10 Endophytic microorganisms from *Azadarichata indica* and *Parthenium hysterophorus (Mane et al.,* 2017*).* They reported anti-tuberculosis activity of isolates against *Mycobacterium tuberculosis* by agar diffusion method.

Table 4: Medicinal plants and their applications

Sr. No	Medicinal plants	Applications
1	Azadarichata indica	Anti-mycobacterial Activity
2	Terminalia arjuna	Heart aliments
3	Aegle marmelos	Antimicrobial activity
4	Catharanthus roseus	Anticarcinogenic activity
5	Dendryphion nanum	Anti-inflammatory and anti-diabetic activity
6	Ocimum sanctum	Antimicrobial activity
7	Shrubby medicinal plants	Isolation of endophytes
8	Licuala ramsayi	New fungal endophytes
9	Camptotheca acuminate	Anti-leukemic and anti-tumour activity
10	Duboisia myoporoides	Production of Tropane alkaloids
11	Taxol	Anticancer activity
12	Camptotheca acuminate	Anti- HIV Activity
13	Artemisia annua	Anti- Malarial activity
14	Phyllanthus amarus	Anti- Hepatitis B activity
15	Dioscorea	Synthesis of Steroidal activity
16	Rauvolfia scrpentina	Alkaloids production
17	Parthenium hysterophorus	Anti- mycobacterial Activity
18	Alove Vera	Antimicrobial & Antifungal activity
19	Withania somnifera	Anti- cancer activity

Terminalia Arjuna Wight & Arn., extensively used against heart ailments in India was studied for Endophytic assemblages of inner bark and twigs [40]. Endophytes ate the chemical synthesizers inside the plant [26]. Peterson *et al.*, (2005) studied endophytic fungal community from different parts of plant *Aegle marmelos* (L.) *Correa* collected from Varanasi (India [22] isolated and purified 52 Endophytic fungi from the leaves of *Catharanthus roseus* plants growing in different regions of Pune, Maharashtra. They screened Endophytic fungi for vinca alkaloid production, purification and their application as an anticarcinogenic agent [12]. studied anti-inflammatory and antidiabetic naphthaquinones from

Dendryphion nanum S. Hughes from the leaves of *Ficus religiosa* collected from Goregaon, Mumbai region (ref). [27] reported Endophytic fungi from 15 shrubby medicinal plants growing in Malanad region, Southern India. She also reported that isolation of more number of Endophytic fungi in winter season rather than in mansoon and summer season. Rodriguez (2008) described a new species *Idriella licualae K.F. Rodrigues and Samuels* from a tropical palm tree, *Licuala ramsayi* [42] reported *Colletotrichum yunnanense* Xiao Ying Liu & W.P. Wu as an Endophytic species isolated from *Baxus* sp. from China

5.3 Fungal Endophytes in Mangroves

In India less reports are available on Endophytic fungi thriving in mangrove plants. Reported fungal endophytes from two species of *Rhizophora*. Further he reported *Acanthus ilicifolius* Linn. *Arthrocnemum undocumented*, *Suaeda maritima* (L) *Dumort* and *Sesuvium portulacastrum* (L) from mangrove plants. [1] reported Endophytic fungi from the roots of mangrove species of west coast region of India. [35] reported cell wall enzymes such as pectate transeliminase, pectinase and protease from Endophytic fungi and their leaf litter degradation activity when exposed to extreme conditions in terrestrial and marine environments such as high temperatures at the tropical areas, elevated hydrostatic pressure, low temperatures in the deep sea, and elevated hydrostatic pressure etc. [30, 35]. Mangrove plants have shown to adapt to anaerobic soil, muddy saline waters, and brackish tidal activities. Association of Endophytic fungi with mangrove plants confers protection from adverse environmental disasters [41].

5.4 Fungal Endophytes from Sea

Marine region is one of the hot spot for the study of Endophytic fungi. Marine endophytic fungi is divided into two groups, that is, obligate and [30, 35]. Obligate marine Endophytic fungi are those that grows and sporulate exclusively in marine water. While facultative marine Endophytic fungi are those, which grow in freshwater or terrestrial milieus, and can as well grow and possibly also sporulate in the marine environment after some physiological adaptations [30, 22]. The marine plants (Sea grasses, Driftwood etc.), marine invertebrates (sponges, corals, bivalves, and crustaceans), vertebrates (fishes), and inorganic matter (soil, sediments) acts as good resources for the marine Endophytic fungi.

6. Future prospects and conclusion

Fungal Endophytes are an untapped, unappreciated microbial society. Endophytic fungal biology has emerged in many clinical applications with molecular approaches. They mimic the plants function and behaviour and are able to produce secondary metabolites as novel compounds. In the upcoming time, the Fungal Endophytes formulation based bio fertilizers may be used to increase soil fertility and crop yield. The Fungal Endophytes can be used in degradation of plastics, electrical materials etc. by taking help from rDNA technology. The Fungal Endophytes can be used to cure cancer, tuberculosis, Malaria, Diabetes Mellitus etc. The Fungal Endophytes can also be used for different fermentation procedures. Molecular study of Fungal Endophytes may help to enhance the activity of drug research area. Fungal Endophytic Nanoparticles may be used to improve plant growth and plant health with sophisticated techniques. However, over the time researchers should address questions about endophyte biology exploring the procedures to confirm fungal Endophytes and differentiate fungal Endophytes from fungal Epiphytes. Further future prospects may involve solving Questions related to how endophytes communicate with each other in the view of their pathogenicity, the biodiversity of Fungal Endophytes functional classes across environmental gradients, their mechanism of plant biogeographic patterns, evolutionary origins, and habitat adaptations and can fungal endophytes be used by rDNA technology successfully.

In conclusion fungal endophytes are the future research of microbial science. Fungal endophytes have the unique adaptation in the nature. Fungal endophytic sources such as grass, medicinal plants, mangroves and sea are hot spots. These Fungal Endophytes have efficient activity to produce bioactive secondary metabolites with antimicrobial, anticancer, antioxidants, insecticidal and cytotoxic activity. They have already shown to be potent source for the discovery of bioactive compounds, but still new and innovative ideas are needed for natural product based drug discovery to overcome drug resistant problem. For such innovative initiation systematic approaches are essential for the study of fungal endophytes.

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