Research Article

Endophytic Fungal Diversity in Medicinal Plants of Western Ghats, India

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Endophytes constitute an important component of microbial diversity, and in the present investigation, seven plant species with rich ethnobotanical uses representing six families were analyzed for the presence of endophytic fungi from their natural habitats during monsoon (May/June) and winter (November/December) seasons of 2007. Fungal endophytes were isolated from healthy plant parts such as stem, root, rhizome, and inflorescence employing standard isolation methods. One thousand five hundred and twenty-nine fungal isolates were obtained from 5200 fragments. Stem fragments harbored more endophytes (80.37%) than roots (19.22%). 31 fungal taxa comprised of coelomycetes (65%), hyphomycetes (32%), and ascomycetes (3%). *Fusarium*, *Acremonium*, *Colletotrichum*, *Chaetomium*, *Myrothecium*, *Phomopsis*, and *Pestalotiopsis* spp. were commonly isolated. Diversity indices differed significantly between the seasons ($P < 0.001$). Species richness was greater for monsoon isolations than winter. Host specificity was observed for few fungal endophytes. UPGMA cluster analysis grouped the endophytes into distinct clusters on the basis of genetic distance. This study is the first report on the diversity and host-specificity of endophytic fungal taxa were from the semi evergreen forest type in Talacauvery subcluster of Western Ghats.

1. Introduction

The microbes residing in the internal parts of plant tissues called “endophytes” constitute a group of plant symbionts and are a component of microbial diversity. Endophytes offer plethora of unknown advantages to the host with immense applications in agriculture and medicine [1, 2]. Recently, challenging hypotheses related to endophyte diversity [3], their role in oxidative stress protection [4], heavy metal tolerance [5], and as components of tropical community ecology [6, 7] have emerged. A perusal of the literature over the past decades indicated many ethnomedicinal plant species with rich botanical history, sampled from unique ecological niches species are known to harbor potential endophytic microbes [8].

There has been an increasing surge of interest among the research groups for the isolation of endophytes from the tropical plant species [9, 10], owing to high plant diversity. One such region represents the Western Ghats, stretching

a length of 1,600 Km from the river Tapti in the state of Gujarat to the Southern tip of Kerala, recognized as one of the 34 hot spots of biodiversity. The Western Ghats represent rich flora with enormous species diversity as well as endemic taxa and are therefore recognized as one among the hot spots of the world [11]. Western Ghats are divided into seven subclusters. A proposal to include and declare 39 sites in this region as the World Natural Heritage Cluster Site by UNESCO is underway (<http://www.atree.org/wg-unesco-whs>).

India has many regions of unique ecological niche harboring variety of medicinal plants. One such region in the peninsular India is Kodagu District, the land of coffee cultivation. Kodagu is situated in the Western Ghats of peninsular India and is known for its majestic mountain ranges, coffee plantations, and teak wood forests. The Talacauvery subcluster (12°17' to 12°27'N and 75°26' to 75°33'E) of the Western Ghats is situated in Kodagu. The altitude ranges from 1525 above mean sea level. Annual precipitation of 3525 mm is largely restricted during May to October,

TABLE 1: Details of medicinal plants collected from the natural habitats of Talacauvery subcluster of Western Ghats.

Plant species*	Common name	Ayurvedic name	Family	Habit	Parts collected
<i>Tylophora asthmatica</i> (W. and A.)	Indian ipecacuanha	Anthrapachaka	Asclepiadaceae	Twiner	Stem
<i>Rubia cordifolia</i> L.	Indian madder	Majith	Rubiaceae	Climber	Stem
<i>Plumbago zeylanica</i> L.	White leadwort	Chitramool	Plumbaginaceae	Shrub	Stem
<i>Phyllanthus amarus</i> (Schum. and Thonn.)	Niruri	Bhoomyamalaki	Euphorbiaceae	Herb	Stem and root
<i>Eryngium foetidum</i> L.	Fit weed/spiny/serrated coriander	Bhandhanya	Apiaceae	Herb	Roots and stem
<i>Centella asiatica</i> L.	Asiatic pennywort	Gotu Kola	Apiaceae	Runner	Stolon, roots, and inflorescence
<i>Zingiber</i> sp.	Wild ginger	Shunti	Zingiberaceae	Herb	Rhizome, aerial stem, and root

Ten plants* were sampled, pooled, and used for isolation of endophytes [13].

although premonsoon showers are not uncommon during February to April. The average temperature is 23°C. Kodagu has a reservoir of forest belts and diverse vegetation ranging from tropical wet evergreen forests to scrub jungles. Several tribes residing in the forests still use medicinal plants of ethnopharmacological importance as the source of natural medication for their ailments [12]. Ethnomedicinal plants are often used in ayurvedic medicinal system in India for the treatment of various diseases.

Despite the reports of ethnomedicinal plants of this region, the biodiversity and the endophytic microbes of this region remain unexplored. Therefore, in the present investigation, seven medicinal plants representing six families were subjected to diversity studies on fungal endophytes during two seasons.

2. Methodology

2.1. Plant Materials and Study Site. Plant parts such as stem, root, rhizome, and inflorescence were collected from seven healthy medicinal plant species: *Tylophora asthmatica*, *Rubia cordifolia*, *Plumbago zeylanica*, *Phyllanthus amarus*, *Eryngium foetidum*, *Centella asiatica*, and *Zingiber* sp. inhabiting the natural vegetation of the Talacauvery Region of Western Ghats, located at 012°17' to 012°27'N and 075°26' to 075°33'E of Kodagu, Karnataka, during the monsoon (May to June) and winter seasons (November–December) of 2007 (Table 1). The natural vegetation is an evergreen/semievergreen type of forests. The mean temperature was 23°C and mean annual precipitation is 3525 mm. Herbarium specimens of the plants were prepared and submitted to the herbarium collections in the DOS in Botany, University of Mysore. Ten individual plants from each were pooled for isolations. The samples were placed in polyethylene bags, labeled, transported in ice box to the laboratory, and placed in a refrigerator at 4°C until isolation. All samples were processed within 24 h of collection.

2.2. Isolation and Identification of Endophytic Fungi. Samples were washed thoroughly in distilled water, blot dried, and first immersed in 70% ethanol (v/v) for one min followed by second immersion in sodium hypochlorite (3.5%, v/v) for three minutes. They were rinsed three times in changes of

sterile distilled water and dried on sterile blotters under the airflow to ensure complete drying. Bits of 1.0 × 0.1 cm size were excised with the help of a sterile blade. A total of 5200 segments from stem, roots, inflorescence, and rhizomes of plant species were placed on water agar (2.5%) supplemented with the antibiotic streptomycin sulphate (100 mg/L). Forty segments were plated per plate. The plates were wrapped in clean wrap cling film and incubated at 22°C with 12 h light and dark cycles for up to 6 to 8 weeks. The effectiveness of surface sterilization of tissues was checked by placing the aliquots of sterilants on agar plates and observing fungal colonies if any for two weeks [14].

Periodically the bits were examined for the appearance of fungal colony and each colony that emerged from segments was transferred to antibiotic-free potato dextrose agar medium (PDA, 2%) to aid identification. The morphological identification of the isolates was done based on the fungal colony morphology and characteristics of the reproductive structures and spores [15–17]. Sporulation was induced by inoculating cultures onto sterilized banana leaf bits (one cm²) impregnated on potato dextrose agar in petri dishes. All fungal mounts were made on microscopic glass slides in lactophenol-cotton blue and sealed with nail polish. Cultures which failed to sporulate were grouped as mycelia sterilia. All the fungal isolates have been catalogued as DST# series with plant code and maintained as culture collections of the department by cryopreservation on PDA overlaid with 15% glycerol (v/v) at –20°C in a deep freezer.

2.3. Data Analysis. Isolation rate (IR), the measure of fungal richness of a sample, was calculated as the number of isolates obtained from tissue segments, divided by the total number of segments, and expressed as fractions but not as percentages [18]. The colonization frequency (CF), expressed as percentage, was calculated according to Kumaresan and Suryanarayanan [19] as follows:

$$\%CF =$$

$$\frac{\text{Number of tissue segments colonized by a fungus}}{\text{Total number of tissue segments plated}} \times 100. \quad (1)$$

FIGURE 1: Relative seasonal isolations of fungal endophytes from plant parts of medicinal species.

The percentage of dominant endophytes (D) was calculated based on the %CF divided by the total number of endophytes $\times 100$ [20].

Differences in the extent of colonization of the samples were analyzed by univariate analysis of variance (one-way ANOVA) and Tukey's honestly significant difference (HSD) as post hoc test using the statistical software SPSS16.0. The fungal isolations were considered for analysis of ANOVA and Tukey's HSD. Simpson and Shannon diversity indices were calculated for endophytic fungi from different seasons with Estimate S, software (version 6, <http://viceroi.eeb.uconn.edu/estimates/>). Species richness was calculated using the online web page rarefactor calculator (<http://www2.biology.ualberta.ca/jbrzusto/rarefact.php>).

Rarefaction indices were employed to compare the species richness among the plant species during two seasons. The expected number of species in N isolations was calculated [21]. Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis was applied for all the isolates from plant species based on the number of isolates recovered from each plant species using a dendrogram constructed based on Nei's genetic distances [22] using tools for population genetics analysis (TFPGA) software [23].

3. Results

A total of 1529 isolates were obtained from 5200 tissue fragments from seven medicinal plant species. The extent of endophytes colonization varied in plant parts with stem fragments harboring 80% of endophytic isolates followed by root (19.22%). In other plant parts, colonization was lower. Isolations of endophytes from various plant parts showed greater numbers of endophytes during monsoon than winter (Figure 1). The high isolation rates (IR) of fungal endophytes were recorded as 1.41 to 1.58 for *T. asthmatica* in both seasons, while in *Zingiber* sp., low rates of isolations

FIGURE 2: Seasonal isolation rates of fungal endophytes from medicinal species. Endophytic fungi were isolated from seven medicinal plant species during monsoon and winter seasons, respectively.

were obtained (Figure 2). Thirty-one fungal taxa were identified which consisted of coelomycetes (65%), hyphomycetes (32%), and ascomycete isolations of 3%. The frequency of fungal colonization (%CF) differed among the seven plant species (Table 2). *Fusarium* sp., *Acremonium*, *Chaetomium*, and *Phoma* are some of the endophytes with high colonization frequency. The dominant fungal genera include *Fusarium* spp. (% D = 10.64) and *Acremonium* (% D = 9.48). Few endophytic fungi such as *A. strictum* had wide distributions in host plants and were isolated from most plants with the exception of *Zingiber* sp. and *Plumbago zeylanica*, whereas species of *Fusarium*, *Trichoderma*, *Curvularia*, and *Penicillium* were isolated from more than three plant species.

Host-specificity was observed for few of the fungal endophytes isolated from two of the seven medicinal plants (Table 2). *Colletotrichum dematium*, *Nigrospora oryzae*, *Heinesia rubi*, *Pestalotiopsis guepinii*, and unidentified red pycnidial form were isolated from the stem segments of *T. asthmatica* only, while in *Rubia cordifolia* one endophytic *Periconia* exhibited specificity. *P. islandicum* and *T. viride* were isolated from root segments of *Phyllanthus amarus*.

Diversity indices of fungal endophytes varied within plant species as well as between seasons (Table 3). High Shannon-Weiner diversity index was recorded for *T. asthmatica* (H^1 = 2.60) and *P. amarus* (H^1 = 2.27) during monsoon and winter seasons, whereas low indices were recorded for *E. foetidum* and *Zingiber* during monsoon and winter seasons, respectively. 42% of the total 31 taxa were found in monsoon season, while 55% of them colonized in both seasons. Simpson index ($1/l$) was high for *T. asthmatica* with a richness of 19 fungal species during monsoon season, while *P. amarus* recorded highest richness of species during winter season. Rarefaction curves calculated for the endophytic fungal isolations indicated maximum species richness for *T. asthmatica* and *P. amarus* during monsoon and winter isolations, respectively

TABLE 2: Colonization frequency* of endophytic fungi isolated from plant parts of seven medicinal plant species.

Seasons			Monsoon										Winter										D (%)									
Plant species	Plant Parts		TA	RC	P	PHY		EF	I	St	CA	I	S	Rh	TA	RC	PZ	PHY		EF	S	R	St	CA	I	S	Rh	ZN	R	D (%)		
			Stem	Stem		S	R	S	R	I	R	R			Stem	Stem		S	R	S	R	S	R	R								
Endophytes																																
<i>Acremonium strictum</i>			25.0	16.0	—	10.0	9.0	8.0	9.0	—	2.0	9.0	—	—	12.5	7.5	15.0	—	5.0	2.5	6.0	5.0	3.5	—	—	—	—	—	—	—	9.48	
<i>Alternaria alternata</i>			—	—	—	—	—	—	—	—	—	—	—	—	—	8.5	—	—	—	1.5	—	—	—	—	—	—	—	—	—	—	0.65	
<i>Aspergillus terreus</i>		5.0	11.5	—	5.0	—	—	—	—	—	—	—	—	—	—	—	—	—	7.0	—	—	—	—	—	—	—	—	—	—	—	1.54	
Red pycnidia			5.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.33	
<i>Botryodiplodia theobromae</i>			2.5	7.5	—	2.5	—	—	—	—	—	—	—	—	—	5.0	—	—	2.5	—	1.5	—	—	—	—	—	—	—	—	—	1.41	
<i>Cladosporium herbarum</i>			—	—	—	2.5	—	—	—	—	—	—	—	0.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.20	
<i>Colletotrichum dematium</i>			—	—	—	—	—	—	—	—	—	—	—	—	20.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.31	
<i>C. lindemuthianum</i>			—	—	—	—	—	—	—	—	—	16.0	—	—	10.0	3.5	—	—	—	—	6.0	—	—	—	—	—	—	—	—	—	2.32	
<i>Chaetomium globosum</i>			27.5	—	—	—	—	—	16.0	—	—	—	—	1.0	16.0	—	3.5	—	—	5.0	—	—	—	—	—	—	—	—	—	—	4.51	
<i>Curvularia lunata</i>			—	—	—	2.5	—	—	—	6.0	4.0	—	—	—	—	10.0	—	—	7.5	—	—	—	2.5	—	—	—	—	—	—	—	2.12	
<i>Fusarium</i> sp.			—	—	—	—	1.5	—	—	—	—	—	—	—	—	—	—	—	1.5	—	—	—	—	—	—	—	—	—	—	—	0.20	
<i>F. graminearum</i>			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.69	
<i>F. oxysporum</i>		6.0	7.5	20.0	—	—	—	—	—	1.0	3.5	—	—	—	—	1.5	—	—	—	—	1.0	4.0	—	—	—	—	—	—	—	—	3.01	
<i>F. solani</i>			5.0	10.0	—	—	4.5	—	5.0	—	—	10.0	—	—	—	—	—	—	4.5	—	—	—	—	—	—	—	—	—	—	—	2.81	
<i>F. pseudonygamai</i>			—	—	—	—	—	2.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.20	
<i>F. verticilloides</i>			21.0	—	5.5	10.0	—	7.5	—	—	—	—	2.0	—	—	—	11.0	—	—	—	—	—	—	—	—	—	—	—	—	—	3.73	
<i>Gelasinospora</i> spp.			2.5	—	—	2.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.29	
<i>Heinesia rubi</i>			1.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.10	
<i>Memnoniella</i> spp.			8.5	2.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.69	
<i>Myrothecium verrucaria</i>			3.0	—	7.5	—	—	—	—	—	—	—	—	—	—	—	—	—	6.0	—	—	—	—	—	—	—	—	—	—	—	1.08	
<i>Nigrospora oryzae</i>			5.0	—	—	—	—	—	—	—	—	5.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.33	
<i>Periconia</i> sp.			—	17.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.14	
<i>Pestalotiopsis guepinii</i>			2.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.13	
<i>Pestalotiopsis</i> sp.			—	—	—	5.0	—	—	—	—	—	—	—	—	2.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.59	
<i>Phoma</i> spp.			6.0	10.0	—	—	—	—	—	—	—	—	—	—	9.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.63	
<i>Phomopsis</i> sp.			5.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.39	
<i>Penicillium</i> sp.			10.0	4.5	—	—	2.0	—	1.0	—	3.0	—	—	—	—	—	2.0	5.0	2.5	—	—	1.5	—	—	—	—	—	—	—	—	2.06	
<i>P. islandicum</i>			—	—	—	7.5	1.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.55	
<i>Trichoderma harzianum</i>			—	—	7.5	—	—	4.5	3.0	—	—	—	—	1.5	—	—	—	5.0	1.5	—	—	5.0	—	5.0	—	—	2.0	—	—	—	1.96	
<i>T.viride</i>			—	—	—	—	5.0	—	—	—	—	—	—	—	—	—	—	—	1.0	—	—	—	—	—	—	—	—	—	—	—	0.39	
<i>Sphaeronema</i> sp.			2.5	—	—	12.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.82	
<i>Verticillium albo-atrum</i>			—	—	3.5	—	—	—	—	—	—	—	1.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.29	
Pycnidial forms			—	7.5	15.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.47	
Sterile mycelia			6.0	—	3.5	5.0	—	1.0	—	—	—	—	—	—	2.0	—	—	—	4.5	—	—	—	—	—	—	—	—	—	—	—	1.44	
Total			314	191	107	126	46	47	68	2	44	79	0	6	6	10	143	72	63	73	34	18	29	20	25	0	0	6	0	1529		
			314	191	107	172	—	—	117	—	—	123	—	—	22	22	143	72	63	107	47	—	—	—	—	—	—	—	—	—	1529	

*200 segments were plated for frequency analysis during monsoon and winter seasons; TA: *Tylophora ashmatica*; RC: *Rubia cordifolia*; PZ: *Plumbago zeylanica*; PHY: *Phyllanthus amarus*; EF: *Eryngium foetidum*; CA: *Centella asiatica*; ZN: *Zingiber* sp.; S: stem; St: stolon; R: root; Rh: rhizome; I: inflorescence.

TABLE 3: Diversity indices (H^1) and species richness of the medicinal plants.

Plant species	Monsoon				Winter			
	Total isolates	Total species richness	Diversity indices		Total isolates	Total species richness	Diversity indices	
			Simpson	Shannon			Simpson	Shannon
<i>Tylophora asthmatica</i>	314	19	0.90	2.60	143	7.0	0.78	1.66
<i>Rubia cordifolia</i>	191	9	0.86	2.05	72	6.0	0.79	1.62
<i>Plumbago zeylanica</i>	107	8	0.84	1.96	63	4	0.63	1.14
<i>Phyllanthus amarus</i>	172	14	0.87	2.29	107	11.0	0.89	2.27
<i>Eryngium foetidum</i>	117	9	0.77	1.68	47	6	0.75	1.53
<i>Centella asiatica</i>	123	8	0.83	1.88	45	6	0.76	1.57
<i>Zingiber</i> sp.	22	8	0.79	1.81	6	2	0.44	0.64

FIGURE 3: Rarefaction curves of fungal endophytes from monsoon isolations (number of isolations versus expected number of species $E(s)$). TA: *Tylophora asthmatica*; RC: *Rubia cordifolia*; PZ: *Plumbago zeylanica*; PHY: *Phyllanthus amarus*; EF: *Eryngium foetidum*; CA: *Centella asiatica*; and ZN: *Zingiber* sp.

(Figures 3 and 4). Differences in the number of isolates and colonization frequency differed significantly between seasons ($P < 0.001$) as indicated in Table 4.

Nei's genetic distance between endophytes isolated from plant species ranged from 0.3185 (between populations of *Zingiber* and *C. asiatica*) to 0.9116 (between populations of *Zingiber* and *T. asthmatica*) (Table 5) which was widely ranged. This indicates a closer relationship between endophytic fungal patterns of both plants. In general, fungal species from *T. asthmatica* is most distanced from the other

FIGURE 4: Rarefaction curves of fungal endophytes from winter isolations (number of isolations versus expected number of species $E(s)$). TA: *Tylophora asthmatica*; RC: *Rubia cordifolia*; PZ: *Plumbago zeylanica*; PHY: *Phyllanthus amarus*; EF: *Eryngium foetidum*; CA: *Centella asiatica*; and ZN: *Zingiber* sp.

plants studied. In order to represent the relationships among plant species, cluster analysis (UPGMA) was used to generate a dendrogram based on Nei's genetic distances between populations (Figure 5). In this dendrogram, all plants form a distinct cluster. When the transect line was placed at approximately 0.4 on the distance scale, two distinct groups were formed. The first cluster was formed by CA-ZN, while

TABLE 4: ANOVA table of seasonal variation of endophytic fungi analyzed from seven medicinal plant species.

	Sum of squares	Degrees of freedom	Mean ²	F	Significance
Between seasons	11931.9	26	458.919	34.382	0.001
Within seasons	1001.062	75	13.346	—	—
Total	12932.96	101	—	—	—

TABLE 5: Nei's genetic distance of plant species analyzed for endophytic fungi.

	TA	RC	PZ	PHY	EF	CA	ZN
TA	***	0.7885	0.5008	0.6061	0.4055	0.7885	0.9116
RC		***	0.5008	0.7239	0.4055	0.3610	0.6061
PZ			***	0.6633	0.3610	0.5008	0.4520
PHY				***	0.4520	0.5008	0.6633
EF					***	0.4055	0.3610
CA						***	0.3185
ZN							***

* Indicates that genetic distance between the same genus does not exist, it is shown for different genera.

FIGURE 5: UPGMA cluster analysis of fungal endophytes representing seven medicinal plant species. Plant codes are TA: *Tylophora asthmatica*; RC: *Rubia cordifolia*; PZ: *Plumbago zeylanica*; PHY: *Phyllanthus amarus*; EF: *Eryngium foetidum*; CA: *Centella asiatica*; and ZN: *Zingiber* sp.

PZ-EF formed the second cluster. PHY, RC, and TA are found outside the cluster.

4. Discussion

Medicinal plants are considered as a repository of “endophytic microbes” living in the internal tissues of plants. The quest for identifying novel bioactives from the endophytic fungi has resulted in the sampling of host plants such as herbs, shrubs, tree species, and vines in unique places of ecological adaptations around the rainforests of the world. Such niches harbor great species diversity, uninvolved by

human activities. Efforts in this direction to sample plants located in the rainforests around the world with potential ethnomedicinal values have resulted in the isolation of fungal endophytes, unique to a particular plant species with distinct bioactivity.

4.1. Endophyte Colonization in Medicinal Species. The medicinal plant species were sampled from the Talacauvery sub-cluster situated in the Kodagu District of Western Ghats of Southern India. This region is among one of the 34 hot spots of biodiversity. Recently, a proposal to include this biodiversity spot in the list of UNESCO Heritage cluster site is underway (<http://www.atree.org/wg.unesco.whs>). The natives as well as the ethnic tribes inhabiting this region still depend on the plants as a source of medicine to cure some of the ailments [12]. Seven medicinal plant species assigned to six plant families were selected for the study in natural populations in two seasons from a single location from the study area stretching over an area of 25 kilometers. Sampling was conducted during monsoon and winter seasons, as two of the herbaceous species, *E. foetidum* and *Zingiber* sp., grow only till the second half of the year (June to December) and their nonavailability during summer (March to May) makes it difficult to consider the summer season for endophytic analysis.

From 5200 segments of plant materials a total of 1529 isolates were obtained; these were grouped into 31 taxa. Mycelia sterilia, the fungal taxa that failed to sporulate, were also reported from this study. This fungal group is prevalent in endophytic studies [24]. The fungal endophytes were analyzed from four plant parts, namely, stem, root, rhizome, and inflorescence; however, their occurrence in root and inflorescence was investigated for few plant species only, as the phenology and sampling of plants never correlated with seasons. The leaves were not considered for isolations since some of the plants were climbers and stragglers with delicate hairy surfaces and stringent surface sterilization techniques would render them unsuitable for plating on agar medium. Relative percentages of endophytic isolations from stem segments were greater (80.37%) than isolations from roots (19.22%). Our results are supported by the earlier work of Huang et al. [25] on 29 traditional Chinese medicinal plants that fungal endophytes are more frequent in stem tissues than roots. Among the fungal taxa, coelomycete isolations were more dominant than hyphomycetes and have been found in earlier studies in endophytes of tree species [26].

Endophytes such as *Colletotrichum*, *Phoma*, *Acremonium*, *Chaetomium*, *Botryodiplodia*, and *Trichoderma* were isolated with %D > 2.0. Few fungal taxa that are less frequently isolated are *Pestalotiopsis*, *Penicillium islandicum*, *Cladosporium herbarum*, *Alternaria alternata*, *F. graminearum*, *Phomopsis*,

and *Sphaeronema*. *Colletotrichum* spp. are the most frequently encountered endophytic fungi [18], whereas *Pestalotiopsis* spp. are well documented as endophytes of many rain-forest plants [27, 28], tropical tree species, namely, *Terminalia arjuna* [29], *Azadirachta indica* [30], and many herbs and shrubs [25, 31, 32]. It is necessary to screen newer plant species for the isolation of fungal endophytes, as Hawksworth and Rossman [33] estimate that there are still millions of species of fungi yet to be identified. Differences in the colonization frequencies of endophytes during two seasons were observed and more isolation during monsoon season is due to the fact that the slimy conidia of fungal spores are dispersed better by rain splashes and germination of conidia is influenced by climatic factors [34].

4.2. Host-Specificity of Fungal Endophytes. We observed that some fungal taxa exhibited host-specificity, a phenomenon often associated with endophytes. Three plant species, *T. asthmatica*, *R. cordifolia*, and *P. amarus*, were host-specific to endophytes. The red pycnidial endophyte (TA-005) was isolated from the stem fragments of *T. asthmatica* only, suggesting the host-specificity of this endophyte. *Pestalotiopsis guepinii* was isolated from the stem segments of *T. asthmatica*. It has been reported as an endophyte of *Wollemia nobilis*, growing in Sydney, Australia [35]. *Heinesia rubi*, *P. islandicum*, and TA-005 are new reports of fungi as endophytes. Host-specificity of endophytic fungi has been observed earlier for grasses [36], orchids [37], and forest tree species [38, 39]. Recently, Sun et al. [40] reiterated the term “host-specificity” as taxa that occur exclusively on a stated host but not on other hosts in the same habitat [41]. Our studies also indicate the host-specificity of endophytes as the plant species were sampled from a single habitat.

4.3. Seasonal Diversity of Fungal Endophytes. Diversity indices for fungal endophytes as analyzed by Shannon-Weiner (H^1) and Simpson ($1/l$) indices indicated differences in seasonal variation and species richness. High indices were noted for *T. asthmatica* ($H^1 = 2.6$) and *P. amarus* ($H^1 = 2.27$) during monsoon and winter seasons, respectively. The fungal species did not differ significantly between plant species, whereas they differed between seasons ($P < 0.001$). Seasonal variation in fungal isolates and colonization frequency has been reported for many host plants [42, 43]. High colonization frequency as well as the species richness of endophytic fungi is limited to leaf segments rather than stem or bark segments of host plants sampled from five medicinal species of Kudremukh Region of Western Ghats [44]. Species richness in our study is limited to stem fragments among the plant parts considered for analysis.

Most studies on fungal endophytes in tropics have revealed remarkable patterns of endophyte colonization and estimates of diversity in foliages of forest tree species representing various sites such as Panamanian Forest [45] and Iwokrama Forest Reserve, Guyana [39]. In the Nilgiri Biosphere Reserve, Western Ghats, India, 75 dicotyledonous species in three different tropical forest types were sampled to study foliar endophytes and diversity [10]. The endophyte

diversity in forest types was limited due to loose host affiliations among endophytes. Studies on foliar endophytes from the sampling of herbaceous and shrubby medicinal plant species have revealed differences in the colonization rates as well as seasonal diversity in Malnad Region of Bhadra Wildlife Sanctuary in Southern India [32, 46].

The present study provides firsthand information on the diversity and seasonal influence on the colonization frequencies of endophytic fungi from selected medicinal plants from one of the subclusters of biodiversity hot spots in the Western Ghats of Southern India. Although the isolation and analysis of endophyte communities in herbs, shrubs, and trees are not uncommon, each of the studies is unique with reference to number of hosts, species of fungal endophytes, and their specificity. The fungal endophytes have been subjected to fermentation studies, and extracts are being tested for biological activities.

5. Conclusion

The study provides firsthand information on the diversity and seasonal influence on the colonization frequencies of endophytic fungi from seven medicinal plants from one of the subclusters of biodiversity hot spots in the Western Ghats of Southern India. The present investigation is the first isolation of endophytes from the medicinal species and their plant parts. Though isolation of endophytes has been accomplished from various forest types and locations around the globe, each study is unique in documenting newer endophytic taxa. We are currently working on the fermentation of fungal endophytes to obtain newer antioxidants with therapeutic applications.

Conflict of Interests

The authors declare no conflict of interests regarding the publication of this paper.

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