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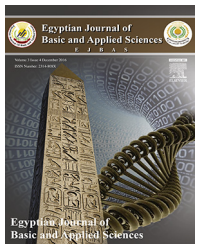

Full Length Article

Diversity and extracellular enzyme activities of fungal endophytes isolated from medicinal plants of Western Ghats, Karnataka

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ABSTRACT

A total of 112 endophytic fungi belonging to 26 genera were isolated from six wild medicinal plants belonging to Bisle region, Western Ghats of Karnataka, among which *Hedychium flavescens* Carey ex Roscoe and *Hedychium coronarium* J. Koenig are listed as endangered plants in the Red data book. The endophytic fungal diversity and extracellular enzyme activity from the endangered plants are reported for the first time. The diversity of the fungal isolates was analyzed using Simpson's diversity indices, Shannon–Weiner index and Evenness. The fungal isolates were screened for the production of extracellular enzymes, of which 29% were positive for amylase, 28% for cellulase, 18% for pectinase and 40% for asparaginase activity. None of the endophytic isolates depicted laccase activity.

1. Introduction

Endophytic fungal communities colonize plants without causing any notable signs of disease to the host plants [1]. They have ubiquitous distribution within plant tissues and are a repository of varied novel compounds with industrial and pharmaceutical potential [2]. Endophytic fungal bioactive compounds from medicinal plants find enormous applications as agrochemicals, antibiotics, antiparasitics, antioxidants, biopesticides and anticancer agents [3]. Endophytes improve the host plant's resistance to adversity by secretion of bioactive metabolites [4]. The diversity of fungal community exists within the tissues of the host plant and among the geographically separated individuals of the same host species [5]. Variation in the fungal diversity may also be associated with location, climate and plant age [6,7]. Diversity analysis of the endophytic fungal assemblages is an emerging challenge, which leads to the discovery of new species producing novel compounds and a better understanding of their role in ecosystems [8]. Recently, fungal

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endophytes have been explored for diverse applications owing to their production of extracellular enzymes [9]. The enzymes function so as to obtain nutrition from their host, hydrolyze food substances and are involved in eliciting defense mechanisms against pathogens [10]. There is an imperative need to discover and utilize diverse novel enzymes with high stability for industrial processes. The present work aims to examine the diversity of endophytic fungal assemblages and their ability to produce extracellular enzymes in selected plants.

2. Experiment and methods

2.1. *Source of endophytic fungi*

Endophytic fungi were isolated from fresh and healthy tissues of six wild medicinal plants collected from Bisle region, Western Ghats of Karnataka and identified as *Tinospora cordifolia* (Willd.) Hook. f. & Thomson, *Piper nigrum* L., *Piper longum* L., *Zingiber officinale* Roscoe, *Hedychium coronarium* J. Koenig and *Hedychium flavescens* Carey ex Roscoe. Herbaria of plant samples were deposited to National Ayurveda Dietetics Research Institute (Central Council for Research in Ayurveda and Siddha), Department of AYUSH, Ministry of Health and Family Welfare, Govt. of India, (New Delhi) Jayanagar, Bangalore, India. The medicinal uses of the plants are listed in Table 1.

2.2. *Isolation and morphological identification of endophytic fungi*

Standard protocols have been followed for the isolation of endophytic fungi as reported in our previous work [11]. The endophytic fungi were identified based on the cultural characteristics, morphology of the fruiting bodies and spores, using standard manuals [12].

2.3. *Frequency of endophytic fungi*

The absolute frequency (f) was calculated as the total number of endophytes isolated [13]. Relative frequencies (fr) of isolation used to represent fungal species density were calculated as the number of isolates of each species of the endophytic fungi divided by the total number of isolates and expressed in percentage. Isolation rate (IR) of the endophytic fungi was calculated as number of isolates obtained from tissue segments divided by total number of tissue segments [14]. The colonization rate (CR) of endophytic fungi was expressed as percentage of total number of isolates obtained from different tissue segments divided by total number of isolates obtained from overall tissue segments incubated [15].

$$\mathrm{IR} = \frac{\text{number of isolates obtained from tissue segments}}{\text{total number of tissue segments}}$$

$$\mathrm{CR} = \frac{\text{total number of isolates obtained from different tissue segments}}{\text{total number of isolates obtained from overall tissue segments incubated}}$$

2.4. *Data analyses*

Simpson's diversity index, Simpson's dominance index (D), Species richness (S), Shannon–Wiener index (H) and Evenness (E) were calculated [16,17].

2.5. *Preliminary screening of endophytic fungi for extracellular enzymes*

The endophytic fungal isolates from selected plants were screened for enzyme production by plate assay method and were assessed by placing 5 mm mycelial plugs on solid media with substrates specific to the respective enzymes: starch to test amylase, hexadecyl trimethyl ammonium bromide to test pectinase, carboxy methyl cellulose to test cellulase, 1-napthol to test laccase and L-asparagine to test for asparaginase. After incubation at room temperature for 7 days, the enzyme activity was determined [18,19].

Table 1 – List of selected medicinal plants and their uses

Sl. No	Medicinal Plant	Family	Herbarium Accession no.	Medicinal uses of the plants
1	*Tinospora cordifolia* (Willd.) Hook. f. & Thomson	*Menispermaceae*	RRCBI-8976	Antipyretic and anti-asthmatic.
2	*Piper nigrum* L.	*Piperaceae*	RRCBI-MUS135	Treatment of vertigo, asthma and sinusitis.
3	*Piper longum* L.	*Piperaceae*	RRCBI-AP -2591	Arthritis and respiratory tract infections.
4	*Zingiber officinale* Roscoe	*Zingiberaceae*	RRCBI-AP.4046	Throat infections, common colds & fever.
5	*Hedychium coronarium* J. Koenig	*Zingiberaceae*	RRCBI-7331	Nausea, halitosis, vomiting, and stimulant
6	*Hedychium flavescens* Carey ex Roscoe	*Zingiberaceae*	RRCBI-MUS.145	Anti-rheumatic, stimulant and antipyretic

2.6. *Statistical analysis*

The experiments were performed in triplicate and the means were analyzed statistically with the SPSS program version 20. The analyses of variance were according to the rules of the ANOVA. The significant differences between the means were determined through Duncan's multiple range Test (DMRT).

3. Results

3.1. *Endophytic colonization in medicinal plants*

A total of 112 isolates were obtained from 480 tissue fragments grouped into twenty six fungal taxa consisting of hyphomycetes (38.4%), ascomycetes (34.6%), coelomycetes (15.38%) and zygomycetes (7.69%) based on their morphology. The extent of colonization varied among the different plant parts with the leaves harboring more endophytic fungi than the other tissues. The isolation rates (IR) was high for *T. cordifolia* whereas low IR was observed for *P. nigrum* and *Z. officinale*. The fungal colonization rate (CR) differed among the six plant species (Table 2). The fungal taxa *Aspergillus, Alternaria, Cladosporium, Colletotrichum, Fusarium, Mucor, Penicillium, Rhizopus* and Mycelia sterilia had high relative frequency of occurrence but fungal taxa *Acremonium, Cylindrocephalum, Dreshclera, Lasiodiplodia, Myrothecium, Nigrospora, Paecilomyces* and *Torula* were found in less frequency (Table 3). All the plant samples were found to be associated with various endophytic fungi with different isolation rates (IR) and colonization rates (CR). The absolute and relative frequencies of occurrence of each endophytic fungal species are depicted in Table 3.

Table 2 – Colonization and isolation rates of endophytic fungi in six medicinal plants.

Sl. no	Medicinal plant	Tissue segments	Colonization rate CR (%)	Isolation rate (IR)
1	*Tinospora cordifolia* (Willd.) Hook. f. & Thomson	Leaf	32	0.4
		Petiole	12	0.15
		Stem	36	0.45
		Roots	20	0.25
2	*Piper nigrum* L.	Leaf	15.38	0.1
		Petiole	15.38	0.1
		Stem	30.76	0.2
		Roots	38.46	0.25
3	*Piper longum* L.	Leaf	40.74	0.55
		Petiole	22.22	0.3
		Stem	29.62	0.4
		Roots	7.40	0.1
4	*Zingiber officinale* Roscoe	Leaf	35.71	0.25
		Petiole	14.28	0.1
		Stem	28.57	0.2
		Roots	21.42	0.15
5	*Hedychium coronarium* J. Koenig	Leaf	40	0.3
		Petiole	26.66	0.2
		Stem	20	0.15
		Roots	13.33	0.1
6	*Hedychium flavescens* Carey ex Roscoe	Leaf	44.44	0.4
		Petiole	27.77	0.25
		Stem	16.66	0.15
		Roots	11.11	0.1

3.1.1. T. cordifolia

The 25 endophytic fungi were isolated from 80 tissue segments of *T. cordifolia*, of which 8 fungal isolates were obtained from leaves, 3 fungal isolates from petiole, 9 fungal isolates from stem and 5 fungal isolates were from roots. They were grouped into 11 genera, of which *Aspergillus* sp. (6.22%), *Cladosporium* sp. (2.67%), Mycelia sterilia (3.56%), and *Fusarium* sp. (2.67%) were the most common and obtained from more than one tissue type (Table 3). The colonization and isolation rates were higher in stem tissues (36%) and leaves (32%) than in the other tissues (Table 2). Simpson and Shannon–Wiener's diversity indices were higher in stem and leaves. The species richness was also greater in the stem and leaves. There was little difference in species evenness among the tissues studied (Table 4).

3.1.2. P. nigrum

A total of 13 endophytic fungal isolates were obtained from 80 tissue segments of *P. nigrum*. Among them, 2 fungal isolates were from leaves, 2 fungal isolates were from petiole, 4 fungal isolates were from stem and 5 fungal isolates were from roots. They were grouped into 9 genera. The most frequently occurring fungal endophytes were *Aspergillus* sp. (3.56%) and *Phoma* sp. (1.78%) (Table 3).The richness of endophytic fungi varied in different tissues of *P. nigrum*. The Simpson and Shannon–Wiener's diversity indices were higher in stem and roots. The species richness was also greater in the roots and stem (Table 4).

3.1.3. P. longum

Twenty seven isolates from 80 tissue segments of *P. longum* were obtained, from which 11 fungal isolates were from leaves, 6 fungal isolates were from petiole, 8 fungal isolates were from stem, and 2 fungal isolates were from roots; these were grouped into 13 genera, in which *Aspergillus* sp., *Penicillium* sp., and Mycelia sterilia were the most commonly occurring and were obtained from more than one tissue type. The frequencies of occurrence of these endophytes were 7.13%, 3.56% and 2.67% respectively (Table 3).The colonization rate was highest in leaves (40.74%), followed by stem (29.62%) (Table 2). The Simpson and Shannon–Wiener's diversity indices were higher in leaves and petiole. The species richness was greater in the leaves (Table 4).

3.1.4. Z. officinale

Fourteen isolates, from which 5 fungal isolates were from leaves, 2 fungal isolates were from petiole, 4 fungal isolates were from rhizome and 3 fungal isolates were from adventitious roots, were obtained from *Z. officinale* and grouped into 11 genera. *Cladosporium* sp. (1.78%) was the most frequently isolated endophyte from only one tissue type (Leaf). Except *Cladosporium* sp., the remaining endophytic fungi had a low frequency of occurrence that is obtained only once from each tissue type (petiole, rhizome and adventitious roots) (Table 3).The colonization rates were higher in rhizomes (36%), leaves (35.71%) and then in the other tissues of *Z. officinale* (Table 2). The richness of endophytic fungi varied among the different tissues of *Z. officinale*. The Simpson's dominance of endophytic fungi was

Table 3 – Absolute (f) and relative frequency (fr) of endophytic fungal isolates from selected medicinal plants.

Sl. no	Endophytic fungi	*Tinospora cordifolia*				*Piper nigrum*				*Piper longum*				*Zingiber officinale*				*Hedychium flavescens*				*Hedychium coronarium*				Absolute frequency (f)	Relative frequency fr (%)
		L	P	S	R	L	P	S	R	L	P	S	R	L	P	S	R	L	P	S	R	L	P	S	R		
1	*Acremonium* sp.															1										1	0.892
2	*Alternaria* sp.													1			1		1	1	1				1	6	5.357
3	*Aspergillus* sp.	3	1	3				2	2	3	1	3	1			1							2			22	19.642
4	*Bipolaris* sp.									1								1						1		3	2.678
5	*Chaetomium* sp.																	1								1	0.892
6	*Cladosporium* sp.		1	2										2						1						6	5.357
7	*Colletotrichum* sp.				1			1		1						1		1	1							6	5.357
8	*Curvularia* sp.			1						1		1			1											4	3.571
9	*Cylindrocephalum* sp.																							1		1	0.892
10	*Dreshclera* sp.									1																1	0.892
11	*Fusarium* sp.	1		1	1							1		1				1		1			1			8	7.142
12	*Lasiodiplodia* sp.							1																		1	0.892
13	*Mucor* sp.	1			1					1		1					1		1							6	5.357
14	Mycelia sterilia	1	1	1	1		1				1	1	1				1		1			1				11	9.821
15	*Myrothecium* sp.																					1				1	0.892
16	*Nigrospora* sp.																						1	1		2	1.785
17	*Paecilomyces* sp.					1																				1	0.892
18	*Penicillium* sp.	1							1	2	1	1										1			1	8	7.142
19	*Pestalotiopsis* sp.										1					1		1								3	2.678
20	*Phoma* sp.					1	1																			2	1.785
21	*Phomopsis* sp.				1						1											1				3	2.678
22	*Pithomyces* sp.									1								1								2	1.785
23	*Rhizopus* sp.	1												1	1			1	1		1					6	5.357
24	*Sordaria* sp.								1		1															2	1.785
25	*Torula* sp.																	1								1	0.892
26	*Trichoderma* sp.			1					1													2				4	3.571
	TOTAL	8	3	9	5	2	2	4	5	11	6	8	2	5	2	4	3	8	5	3	2	6	4	3	2	112	99.984
	Species richness	6	3	6	5	2	2	3	4	8	6	6	2	4	2	4	3	8	5	3	2	5	3	3	2		

L, Leaf; P, Petiole; S, Stem; R, Root.

Table 4 – Diversity indices of endophytic fungi isolated from *T. cordifolia*, *P. nigrum* and *P. longum*.

Sl. No	Indices	*Tinospora cordifolia*				*Piper nigrum*				*Piper longum*			
		L	P	S	R	L	P	S	R	L	P	S	R
1	Simpson's dominance	0.218	0.333	0.209	0.200	0.500	0.500	0.375	0.280	0.157	0.166	0.218	0.500
2	Simpson's diversity	0.781	0.666	0.790	0.800	0.500	0.500	0.625	0.720	0.843	0.833	0.781	0.500
3	Species Richness	6	3	6	5	2	2	3	4	8	6	6	2
4	Shannon–Wiener	1.667	1.097	1.676	1.609	0.693	0.693	1.039	1.331	1.971	1.791	1.667	0.693
5	Evenness	0.930	0.998	0.935	0.999	0.999	0.999	0.946	0.960	0.948	0.999	0.930	0.999

L, Leaf; P, Petiole; S, stem; R, root.

higher in the petiole. The Shannon–Wiener's diversity indices were higher in leaves and rhizomes (Table 5).

3.1.5. H. flavescens

A total of 18 endophytic fungal isolates were attained from 80 tissue segments of *H. flavescens* (8 fungal isolates from leaves, 5 fungal isolates from petiole, 3 fungal isolates from stem and 2 fungal isolates from roots) and were grouped into 12 genera. The species of *Alternaria* and *Rhizopus* occurred in low frequency of 2.67% (Table 3). The colonization rate was highest in leaves (44.44%) and petiole (27.77%) than in the other tissues of *H. flavescens* (Table 2). The species richness was high in the leaves followed by petiole (Table 5).The Simpson's dominance of endophytic fungi was higher in the roots. Both Simpson and Shannon–Wiener's diversity indices were higher in leaves and petiole.

3.1.6. H. coronarium

Fifteen endophytic isolates were obtained – 6 fungal isolates from leaves, 4 fungal isolates from petiole, 3 fungal isolates from stem and 2 fungal isolates from roots which were grouped into 12 genera. The colonization rates were highest in leaves (40%) and petiole (26.6%) (Table 2). The Simpson and Shannon–Wiener's diversity indices were higher in leaves. The species richness was also greater in the leaves (Table 5). The genera *Aspergillus* and *Cladosporium* were the most frequently isolated in the petiole and leaf tissues (Table 3).

3.1.7. *Screening of the endophytic fungi for extracellular enzyme production*

The fungal isolates were subjected for extracellular enzyme production. Eighty isolates were able to produce the extracellular enzymes (Table 6) with the exception of laccase which none of the fungal isolates produced. The incubation period influenced enzyme production and varied from 3 to 7 days. In our study, 29% of the isolates hydrolyzed starch and were positive for amylase activity. The maximum production of amylase was from Pn-7 (*Lasiodiplodia* sp.), Pl-13 (*Aspergillus* sp.) and Zo-3 (*Cladosporium* sp.). The isolates of *H. coronarium* and *H. flavescens* were weak producers of amylase (Table 6). Cellulolytic activity was observed in 28.18% of the total isolates. Prominent cellulolytic activity was observed in Tc-8 (Mycelia sterilia), Pl-13 (*Aspergillus* sp.), Pl-10 (*Penicillium* sp.), Pl-21 (*Pestalotiopsis* sp.) and Hc-3 (Mycelia sterilia) (Table 6). The isolates demonstrating pectinase enzyme were lower compared to other enzymes. Maximum pectinase activity was observed in *T. cordifolia* isolates (24%). The isolates from *P. nigrum*, *H. coronarium* and *H. flavescens* were weak producers of pectinase. Significant pectinase activity was detected in Tc-8 (Mycelia sterilia), Zo-3 (*Cladosporium* sp.), Pl-24 (*Phomopsis* sp.) and Hf-8 (*Mucor* sp) (Table 6). None of the isolates screened demonstrated laccase activity, indicating the disparity in enzyme production by the fungal species which is mostly dependent on the host habitat. Asparaginase activity was depicted by 39% of the endophytic isolates. The highest L-asparagine producing isolates were observed from *P. longum* where 13 of the 27 isolates produced asparaginase. The isolates Tc-25 (*Fusarium* sp.), Zo-14 (*Rhizopus* sp.) and Zo-7 (*Aspergillus* sp.) demonstrated high asparaginase activity (Table 6).

4. Discussion

The plant tissues, namely the leaf, petiole, stem and roots of the medicinal plants, were used. The members of *Hedychium* (*Zingiberaceae*) are listed as critically endangered species of India in the Red data book [20]. However, not much work has been done on endophytic fungal isolations from the plants belonging to *Hedychium* sp. to the best of our knowledge. Also, the studies on endophytic fungi from these plants have revealed

Table 5 – Diversity indices for endophytic fungi isolated from *Z. officinale*, *H. flavescens* and *H. coronarium*.

Sl. No	Indices	*Zingiber officinale*				*Hedychium flavescens*				*Hedychium coronarium*			
		L	P	Rh	Ad. R	L	P	S	R	L	P	S	R
1	Simpson's dominance	0.280	0.500	0.250	0.333	0.125	0.200	0.333	0.500	0.222	0.370	0.333	0.500
2	Simpson's diversity	0.720	0.500	0.750	0.666	0.875	0.800	0.666	0.500	0.777	0.625	0.666	0.500
3	Species Richness	4	2	4	3	8	5	3	2	5	3	3	2
4	Shannon–Wiener	1.331	0.693	1.386	1.098	2.079	1.604	1.096	0.693	1.560	1.039	1.098	0.693
5	Evenness	0.960	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.969	0.946	0.999	0.999

L, Leaf; P, Petiole; S, stem; R, root; Rh, Rhizome; Ad. R, Adventitious roots.

Table 6 – Endophytic fungal isolates screened for extracellular enzymes on solid media.

Sl. No	Medicinal plant	Code	Endophytic fungal isolate	Amylase	Cellulase	Pectinase	Laccase	Asparaginase
1	*Tinospora cordifolia*	Tc2	*Fusarium* sp.	$29.00^{d} \pm 0.51$	0^{a}	0^{a}	0^{a}	$25.00^{b} \pm 0.59$
2	(Willd.) Hook. f. & Thomson	Tc6	*Aspergillus* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$47.66^{cd} \pm 1.30$
3		Tc7	*Penicillium* sp.	$36.33^{f} \pm 0.59$	0^{a}	0^{a}	0^{a}	$43.66^{cd} \pm 0.78$
4		Tc8	Mycelia sterilia	$34.33^{e} \pm 0.29$	$55.33^{f} \pm 1.07$	$72.33^{f} \pm 0.78$	0^{a}	$34.66^{bc} \pm 0.29$
5		Tc9	*Cladosporium* sp.	0^{a}	$15.33^{c} \pm 0.78$	$69.33^{e} \pm 0.59$	0^{a}	$54.00^{d} \pm 0.51$
6		Tc10	*Aspergillus* sp.	$18.33^{b} \pm 0.78$	$21.66^{d} \pm 0.29$	$41.66^{c} \pm 0.78$	0^{a}	0^{a}
7		Tc12	*Curvularia* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$59.00^{d} \pm 0.51$
8		Tc15	*Trichoderma* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$75.00^{e} \pm 0.29$
9		Tc16	*Cladosporium* sp.	$38.33^{g} \pm 0.78$	$13.66^{b} \pm 0.78$	$41.00^{c} \pm 0.51$	0^{a}	0^{a}
10		Tc17	*Fusarium* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$23.66^{ab} \pm 0.78$
11		Tc18	*Cladosporium* sp.	0^{a}	$21.00^{d} \pm 0.51$	0^{a}	0^{a}	
12		Tc19	*Aspergillus* sp.	0^{a}	$24.00^{e} \pm 0.23$	$53.00^{d} \pm 0.51$	0^{a}	$24.00^{b} \pm 0.78$
13		Tc20	*Aspergillus* sp.	$24.66^{c} \pm 0.29$	$24.00^{e} \pm 0.51$	$39.33^{b} \pm 0.59$	0^{a}	$36.00^{bc} \pm 0.51$
14		Tc25	*Fusarium* sp.	$29.00^{d} \pm 0.51$	0^{a}	0^{a}	0^{a}	$79.66^{e} \pm 0.29$
15	*Piper nigrum*	Pn1	*Phoma* sp.	0^{a}	0^{a}	$9.00^{b} \pm 0.51$	0^{a}	0^{a}
16	L.	Pn2	*Paecilomyces* sp.	0^{a}	$21.6^{6b} \pm 0.29$	0^{a}	0^{a}	0^{a}
17		Pn6	*Aspergillus* sp.	$19.33^{b} \pm 0.29$	0^{a}	0^{a}	0^{a}	$69.66^{e} \pm 0.29$
18		Pn7	*Lasiodiplodia* sp.	$69.00^{d} \pm 0.51$	0^{a}	0^{a}	0^{a}	$65.66^{d} \pm 0.29$
19		Pn8	*Aspergillus* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$34.00^{b} \pm 0.51$
20		Pn9	*Aspergillus* sp.	$49.00^{c} \pm 0.51$	0^{a}	0^{a}	0^{a}	0^{a}
21		Pn11	*Sordaria sp.*	0^{a}	$43.33^{d} \pm 0.29$	0^{a}	0^{a}	0^{a}
22		Pn12	*Aspergillus* sp.	0^{a}	$26.00^{c} \pm 0.51$	$23.66^{c} \pm 0.29$	0^{a}	$69.00^{e} \pm 0.51$
23		Pn13	*Trichoderma* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$64.00^{c} \pm 0.51$
24	*Piper longum*	Pl1	*Mucor* sp.	$44.00^{i} \pm 0.51$	$46.00^{f} \pm 0.51$	0^{a}	0^{a}	$39.00^{c} \pm 0.51$
25	L.	Pl2	*Penicillium* sp.	0^{a}	$49.00^{g} \pm 0.51$	0^{a}	0^{a}	0^{a}
26		Pl3	*Aspergillus* sp.	0^{a}	$32.33^{c} \pm 0.29$	0^{a}	0^{a}	0^{a}
27		Pl4	*Colletotrichum* sp.	0^{a}	$37.00^{e} \pm 0.51$	$10.66^{ab} \pm 0.29$	0^{a}	0^{a}
28		Pl5	*Penicillium* sp.	0^{a}	$34.00^{d} \pm 0.51$	0^{a}	0^{a}	$47.66^{gh} \pm 0.29$
29		Pl7	*Dreshclera* sp.	$31.66^{f} \pm 0.29$	0^{a}	0^{a}	0^{a}	0^{a}
30		Pl8	*Aspergillus* sp.	0^{a}	$49.00^{g} \pm 0.57$	0^{a}	0^{a}	$47.66^{gh} \pm 0.33$
31		Pl9	*Pithomyces* sp.	0^{a}	0^{a}	0^{a}	0^{a}	39.00c ± 0.57
32		Pl10	*Bipolaris* sp.	0^{a}	$54.66^{i} \pm 0.33$	0^{a}	0^{a}	$40.33^{de} \pm 0.33$
33		Pl11	*Curvularia* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$39.66^{cd} \pm 0.29$
34		Pl12	*Fusarium* sp.	0^{a}	$52.33^{h} \pm 0.29$	0^{a}	0^{a}	$39.33^{cd} \pm 0.59$
35		Pl13	*Aspergillus* sp.	$52.66^{j} \pm 0.29$	$71.00^{k} \pm 0.51$	0^{a}	0^{a}	$41.00^{e} \pm 0.51$
36		Pl14	*Curvularia* sp.	$24.00^{bc} \pm 0.51$	0^{a}	0^{a}	0^{a}	0^{a}
37		Pl15	*Aspergillus* sp.	0^{a}	$54.66^{i} \pm 0.29$	0^{a}	0^{a}	$45.00^{f} \pm 0.48$
38		Pl16	*Aspergillus* sp.	0^{a}	0^{a}	$18.33^{b} \pm 0.29$	0^{a}	$24.33^{b} \pm 0.63$
39		Pl17	*Mycelia sterilia*	$29.33^{e} \pm 0.59$	0^{a}	0^{a}	0^{a}	0^{a}
40		Pl18	*Mucor* sp.	$25.66^{d} \pm 0.29$	0^{a}	0^{a}	0^{a}	0^{a}
41		Pl19	*Penicillium* sp.	$36.00^{g} \pm 0.51$	0^{a}	0^{a}	0^{a}	0^{a}
42		Pl20	*Aspergillus* sp.	0^{a}	$54.00^{i} \pm 0.51$	0^{a}	0^{a}	0^{a}
43		Pl21	*Pestalotiopsis* sp.	0^{a}	$56.33^{j} \pm 0.29$	0^{a}	0^{a}	$48.00^{h} \pm 1.03$
44		Pl22	*Mycelia sterilia*	$39.00^{h} \pm 0.51$	0^{a}	0^{a}	0^{a}	0^{a}
45		Pl24	*Phomopsis* sp.	$29.66^{e} \pm 0.29$	$21.66^{d} \pm 0.29$	$61.66^{c} \pm 0.29$	0^{a}	0^{a}
46		Pl25	*Penicillium* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$39.66^{cd} \pm 0.29$
47		Pl26	*Aspergillus* sp.	$23.33^{b} \pm 0.59$	0^{a}	0^{a}	0^{a}	$46.66^{g} \pm 0.29$
48		Pl27	*Mycelia sterilia*	$24.66^{c} \pm 0.29$	0^{a}	$81.00^{d} \pm 0$	0^{a}	0^{a}
49	*Zingiber officinale*	Zo1	*Aspergillus* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$32.00^{b} \pm 0.51$
50	Roscoe	Zo2	*Rhizopus* sp.	$41.00^{d} \pm 0.51$	$40.33^{c} \pm 0.29$	$39.66^{d} \pm 0.29$	0^{a}	0^{a}
51		Zo3	*Cladosporium* sp.	$53.33^{f} \pm 0.78$	0^{a}	$63.33^{e} \pm 0.78$	0^{a}	0^{a}
52		Zo4	*Cladosporium* sp.	$29.00^{c} \pm 0.51$	0^{a}	0^{a}	0^{a}	0^{a}
53		Zo5	*Alternaria* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$63.33^{d} \pm 0.29$
54		Zo6	*Curvularia* sp.	$14.66^{b} \pm 0.29$	0^{a}	0^{a}	0^{a}	0^{a}
55		Zo7	*Aspergillus* sp.	0^{a}	$35.33^{b} \pm 0.29$	0^{a}	0^{a}	$72.33^{e} \pm 0.29$
56		Zo8	Mycelia sterilia	0^{a}	0^{a}	$26.66^{c} \pm 0.29$	0^{a}	0^{a}
57		Zo11	Mycelia sterilia	0^{a}	0^{a}	0^{a}	0^{a}	$41.66^{c} \pm 0.29$
58		Zo13	*Mucor* sp.	$42.66^{e} \pm 0.29$	0^{a}	$10.33^{b} \pm 0.59$	0^{a}	0^{a}
59		Zo14	*Rhizopus* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$72.33^{e} \pm 1.30$
60	*Hedychium coronarium*	Hc2	*Trichoderma* sp.	0^{a}	$26.66^{c} \pm 0.29$	0^{a}	0^{a}	0^{a}
61	J. Koenig	Hc3	Mycelia sterilia	0^{a}	$65.00^{d} \pm 0.51$	0^{a}	0^{a}	$23.66^{c} \pm 0.78$
62		Hc4	*Penicillium* sp.	$29.00^{c} \pm 0.51$	0^{a}	0^{a}	0^{a}	0^{a}
63		Hc5	Mycelia sterilia	0^{a}	0^{a}	0^{a}	0^{a}	$31.00^{e} \pm 0.51$
64		Hc7	*Alternaria* sp.	$11.66^{b} \pm 0.29$	0^{a}	0^{a}	0^{a}	0^{a}
65		Hc8	*Penicillium* sp.	0^{a}	0^{a}	$14.00^{b} \pm 0.51$	0^{a}	$51.00^{g} \pm 0.51$
66		Hc9	*Fusarium* sp.	0^{a}	$20.66^{b} \pm 0.29$	0^{a}	0^{a}	0^{a}
67		Hc11	*Aspergillus* sp.	0^{a}	0^{a}	$19.00^{c} \pm 0.51$	0^{a}	$15.66^{b} \pm 0.29$
68		Hc12	*Bipolaris* sp.	$12.00^{b} \pm 0.51$	0^{a}	0^{a}	0^{a}	0^{a}
69		Hc13	*Nigrospora* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$24.66^{d} \pm 0.29$
70		Hc15	*Aspergillus* sp.	$44.66^{d} \pm 0.29$	0^{a}	0^{a}	0^{a}	$32.66^{f} \pm 0.29$
71	*Hedychium flavescens*	Hf3	*Colletotrichum* sp.	0^{a}	$29.33^{c} \pm 0.29$	$27.33^{c} \pm 0.29$	0^{a}	0^{a}
72	Carey ex Roscoe	Hf4	*Fusarium* sp.	$39.66^{c} \pm 0.29$	0^{a}	0^{a}	0^{a}	$41.33^{d} \pm 0.29$
73		Hf7	*Bipolaris* sp.	0^{a}	0^{a}	0^{a}	0^{a}	$65.00^{e} \pm 1.03$
74		Hf8	*Pithomyces* sp.	$52.66^{d} \pm 0.29$	0^{a}	0^{a}	0^{a}	0^{a}
75		Hf9	*Mucor* sp.	0^{a}	0^{a}	$62.66^{d} \pm 0.29$	0^{a}	0^{a}
76		Hf10	*Alternaria* sp.	0^{a}	$17.66^{b} \pm 0.29$	0^{a}	0^{a}	0^{a}
77		Hf11	Mycelia sterilia	0^{a}	0^{a}	0^{a}	0^{a}	$11.66^{b} \pm 0.29$
78		Hf12	*Rhizopus* sp.	$19.66^{b} \pm 0.29$	0^{a}	0^{a}	0^{a}	0^{a}
79		Hf12	*Fusarium* sp.	0^{a}	0^{a}	$15.66^{b} \pm 0.29$	0^{a}	$23.66^{c} \pm 0.29$
80		Hf14	*Cladosporium* sp.	0^{a}	$42.66^{d} \pm 0.29$	0^{a}	0^{a}	0^{a}

Values followed by lower case alphabets in the column are statistically equivalent ($P < 0.05$) according to the Duncan multiple range test.

differences in the colonization rates as well as diversity pattern which have not been documented so far. Previous report suggests that fungal endophytes were more frequent in leaf and stem tissues [14]. In contrast, in the species composition of endophytic fungi from *Lippia sidoides*, the colonization of leaves (50.41%) was higher than that of stems (35.40%) [21]. In Brazil, 95 endophytic fungi from *Bauhinia forficata* were isolated and reported highest frequency of colonization in the stems [22]. Species of *Aspergillus, Fusarium, Penicillium, Colletotrichum, Cladosporium, Curvularia, Mucor and Rhizopus* were dominant in our work and it may be due to high spore production of these fungi and their cosmopolitan nature, which increases their chance to get established as endophytes [23]. Mycelia sterilia, the fungal taxa which failed to sporulate, are also reported in our present work. The species of *Acremonium, Colletotrichum, Chaetomium, Myrothecium, Phomopsis, Fusarium* and *Pestalotiopsis* were the commonly isolated endophytes from medicinal plants of Western Ghats, Karnataka [24]. There is a dearth of reports on the diversity of endophytic fungi obtained from *Z. officinale* as many authors have worked on the antagonistic activity of the endophytic actinomycetes of *Z. officinale* against phytopathogenic fungi [25].

Diversity indices for fungal endophytes analyzed by Shannon–Weiner and Simpson indices indicated differences in endophytic fungal isolates and species richness. High Simpson's diversity indices were noted for *P. longum* and *H. flavescens* whereas lower diversity indices are reported in *P. nigrum*. The colonization rate was higher in the leaves and stems of the medicinal plants as compared to the roots and petiole. Species richness was predominant in leaves. Species richness was higher in leaf segments of the five medicinal plants of Kudremukh region, Western Ghats of Karnataka which is similar to our work [23].

The Shannon index increases as both the richness and the evenness of the community increase. The Simpson's dominance and diversity were analyzed. As the dominance index increases, the diversity decreases. Species richness relates to count of species, whereas species evenness quantifies the equal abundances of the species in a particular environment. Lesser variation in communities between the species reflects higher species evenness and is independent of species richness.

This study reports *Lasiodiplodia* sp. (Pn-7) as producing maximum amylolytic activity out of all the isolates. From our laboratory, thirty isolates from *Alpinia calcarata* Roscoe were screened, of which the isolate *Cylindrocephalum* sp. gave maximum amylase activity [26]. Among 112 isolates, thirty one isolates have shown cellulolytic activity (27.67%) and the highest cellulolytic activity was demonstrated in *Aspergillus* sp from *P. longum* (Table-6). Previously, cellulolytic activity was prominent in *Talaromyces emersonii* [19]. In another study, 43.33% isolates exhibited cellulase activity with *Cephalosporium* (36.5%) being the prominent cellulase producing form [27]. Moreover, 66% of the isolates from *Brucea javanica* were producers of cellulase enzyme [28]. Pectinase activity was observed in 19% of our endophytic isolates Endophytic fungi from *Opuntia ficus-indica* Mill were isolated, wherein *Cladosporium cladosporioides* (20.43%) and *Cladosporium sphaerospermum* (15.99%) presented high pectinase activity [22]. Maximum pectinase activity was reported in *T. emersonii* and *Fusarium oxysporum* from *Calophyllum inophyllum* [19]. In fungi, laccase is a ligninolytic enzyme and is involved in fruiting body formation, fungal plant-pathogen/host interaction and stress defense. Laccase activity reflects the ability of the fungus to decompose lignocellulosic materials. An interesting observation of our study is that none of the endophytic fungal isolates were able to produce laccase enzyme. Also, none of the endophytic fungi from mangrove angiosperms were able to produce laccase [29]. The endophytic nature of these fungi might be the reason for the lack of laccase activity, since an active enzyme might damage the host plant. The endophytic *Phomopsis longicolla* of *Bixa orellana* was a significant producer of laccase enzyme. In addition, *Discosia* sp. from *C. inophyllum* and *Chaetomium* sp. from *Alpinia calcarata* produced laccase [14]. Asparaginase activity was depicted by 40% of the isolates. The isolate *Fusarium* sp. (Tc-25) gave the highest asparaginase activity followed by *Aspergillus* sp. (Zo-7) and *Rhizopus* sp. (Zo-14). Sixteen endophytic fungi were isolated from *Capsicum frutescence* var US 341 and evaluated for L-asparaginase production. Among them, *Aspergillus* sp. was identified as a potential isolate for L-asparaginase [30]. The endophytic fungal isolates from seven wild Thai medicinal plants were screened for asparaginase, from which *Colletotrichum* sp. and Mycelia sterilia exhibited good asparaginase activity [18].

5. Conclusion

The present study provides information on colonization of endophytic fungi in six important medicinal plants and its diversity analysis. This is a first report on endophytic assemblages from *Hedychium flavescens* and *H. coronarium*. The extracellular enzymes of endophytic fungi varied from isolate to isolate, hypothesizing that the enzyme production depends on the type of host and its habitats. Further research is required for the synthesis of stable enzymes and bioactive compounds.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.ejbas.2016.08.007.

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