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Diversity and biological activities of fungal root endophytes of *Hemidesmus indicus* (L.) R. Br.

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Abstract
The roots of *Hemidesmus indicus* are known to have bioactive properties. Large scale exploitation of plant roots may endanger the plant from ecosystem. Endophytes are known to produce many bioactive compounds as that of host plant. Endophytic fungi residing in various parts of the plant like root, stem and leaves were isolated and identified. Colonization frequency and relative dominance of the endophytes were evaluated. The ethyl acetate extracts of culture broth and mycelial mat was screened for antioxidant and antimicrobial activity.285 fungal isolates from the roots of *H. indicus* were isolated**,** identified and authenticated by molecular tools. The overall colonization frequency was found to be 70.33%, of which hyphomycetes were dominant (79.45%). Species of *Fusarium* were the dominant colonizers of all parts of the plant. Antioxidant activities of ethyl acetate fractions revealed that the extracts of *F. oxysporum* and *A. sydowii* showed higher activities with IC_{50} values of 45 and 46.6 µg/mL respectively. The extracts also exhibited promising antibacterial activities against both Gram positive and Gram negative bacteria. The present study reported for the first time the endophytic fungi from the roots of *H. indicus* and their bioactivity. Our present findings highlight the potential of the isolated endophytes to have therapeutic applications.

Keywords: root endophytes, secondary metabolites, antioxidant activity, antimicrobial activity

Introduction
Plants are the important sources of traditional medicine practised worldwide. The use of plants for therapy has been increased in recent years due to the disadvantages of using synthetic drugs. In most of the medicinal plants, roots are the important sources of phytomedicine. Continued use of plants for their medicinal use may result in gradual extinction from wild. Hence, a substitute for the active molecule other than plant having the similar or enhanced bioactivity is being explored.

All the plants are colonised by mutualistic endophytic microorganisms. Endophytes produce several secondary metabolites which can be used in medicine and agriculture. The compounds derived from these organisms are used as anticancer, antimicrobial, anti-inflammatory, anti-mycobacterial, antioxidant agents [1]. For example, Paclitaxel, a diterpenoid compound obtained from *Taxomyces andreanae* [2] has anticancer properties. Similarly, podophyllotoxin from *Sinopodophyllum hexandrum* has anti-inflammatory and antimicrobial properties [3]. Enfumafungin from *Hormonema* spp. and lovostatin obtained from *Aspergillus luchuensis* helps to reduce cholesterol levels [4, 5]. Species of *Pseudomassaria* are known to synthesize antidiabetic agents [6]. Immunosuppressive agents like subglutinol A and B are obtained from endophytic *Fusarium subglutinans* [7]. *Trichoderma atroviridae* inhabiting the roots of *Salvia miltiorrhiza* is known to produce tanshinone I and IIA that are effective as therapeutic agents [8]

Endophytes that colonize the inner parts of the root are usually diverse in nature, since roots are the zones of maximum interaction with the rhizosphere. Endophytes offer several advantages to the host plant like growth promotion [9], inhibiting the growth of pathogenic fungi and bacteria [10, 11]. Endophytes are helpful in establishing successful symbiosis via altering the production of secondary metabolites. Promising antimicrobial metabolites have been reported from root endophytes of *Acorus calamus* [12]. *Penicillium* spp. isolated from *Panax ginseng* produces brefeldin A, which has antimicrobial properties [13].

Hemidesmus indicus (L.) R. Br. commonly called Indian sarsaparilla, is a member of the family Apocyanaceae. The plant has been perceived as an official drug in Indian and British Pharmacopoeia. The plant is used to treat scorpion bites, snake bites and fever [14]. The decoction of root is used as a diaphoretic, blood purifier, diuretic, refrigerant and tonic. The plant finds its use in treating respiratory disorders, skin diseases, leprosy, leucoderma, leucorrhoea, rheumatism and several other ailments [15]. The Root decoction is useful in

curing high fever and skin diseases [16]. The roots of plant has a strong antioxidant [17], antithrombotic, anti-inflammatory and antiproliferative activities [18]. As large scale harvesting of plants, especially roots, may endanger the plant, alternative sources of similar bioactive compounds are explored.

With this background, our study aimed at the isolation of endophytic fungi residing in different parts of *H. indicus*, especially from roots. Endophytic fungi are reported to produce several bioactive compounds, similar compounds from endophytes residing in the roots of *H. indicus* was investigated. Further, the isolated endophytic fungi were also bioprospected for its ability to act as antioxidant and antimicrobial agents, similar to that of the host.

Materials and Methods

Chemicals

Gallic acid, 2, 2'-azino-bis (3- ethylbenzthiazoline-6-sulphonic acid) (ABTS), and 1, 1-diphenyl-2- picrylhydrazyl (DPPH) were procured from Sigma-Aldrich, Germany. Folin–Ciocalteu's reagent was purchased from S D fine-chemicals Pvt. Ltd. (Mumbai, India). Chloramphenicol, Muller Hinton agar and antibiotic discs were purchased from HiMedia (Mumbai, India). Other general chemicals and solvents of analytical grade were used for the present study.

Identification and collection of plant samples

The plant samples were collected from three different places; Gonikoppal (South Kodagu, Karnataka, India) Chamundi Hills (Mysore, Karnataka, India) and Kirugavalu (Mandya, Karnataka, India). The collected plant samples were identified and confirmed by a plant taxonomist. These were stored in plastic bags, processed within 24-48h of collection and were sampled for the isolation of endophytes.

Isolation of endophytic fungi

The root, stem and leaves of plant were washed thoroughly with tap water to remove the adhering soil particles. The samples were surface sterilized with 70% ethanol for a minute, followed by treatment with 4.5% (v/v) sodium hypochlorite (3% available chlorine) for 3min and rinsed three times with sterile distilled water, one minute each to ensure complete removal of surface sterililants [19]. The process was carried out under aseptic conditions to avoid contamination.

The plant parts were cut into suitable size with the help of a sterile blade, placed in a Petri dish containing sterile water agar media (15 g/L) supplemented with 250ppm Chloramphenicol to inhibit the bacterial growth. The plates were incubated at 28 ^{0}C for 7days in alternate dark and light cycles (16 h/8 h). The endophytic mycelia emerging from the plant tissue were carefully picked, transferred to a sterile potato dextrose agar media to facilitate the growth and sporulation. The endophytes were preserved in cryovials on PDA, overlaid with 15% (v/v) glycerol and stored at -80^{0}C.

Molecular authentication of selected endophytes

The identification of endophytic fungi was primarily done based on their colony characters, spore morphology or fruiting bodies, if any, using standard identification manuals. Endophytic fungi were harvested for DNA extraction by CTAB (cetyl trimethyl ammonium bromide) method as described by Doyle and Doyle [20]. The extracted DNA was stored in 100µL Tris EDTA buffer. The genomic DNA was quantified, checked for its purity in a Nanodrop (NDC 2000 spectrophotometer, Thermo Scientific, Japan). The ratio 260nm to 280nm corresponding to the values between 1.6-1.8 indicated the presence of relatively pure DNA without being contaminated with protein/phenol or RNA. Presence of genomic DNA was further confirmed by running the samples on 1% agarose gel supplemented with ethidium bromide and the bands of DNA was visualised in a gel documentation system (BioRad, California, USA)

The extracted DNA from endophytic fungi was amplified using universal ITS primers for fungi *i.e.* ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) according to White *et al.* [21]. The reaction was carried out in a thermal cycler in 0.2mL PCR tubes containing 25 µL reaction mixture including 1µL of the genomic DNA at a concentration of 50ng/µL. The program involved initial denaturation at 95^{0}C for 5min and 94^{0}C for 3 min, primer annealing at 55^{0}C for 1min and extension at 72^{0}C for 2min and 10min. The program was repeated for 35 cycles. The amplified products (5µL) were loaded on 1% agarose gel and the size was compared with the standard ladder (100-1000bp). The amplified products were sent to Chromous biotech, Bangalore for sequencing. The obtained sequences were analysed using BLAST tool available in National Centre for Biotechnology Information, NCBI. The sequences showing homology to the reference sequences were identified and deposited in GenBank sequence submission system (BankIt, NCBI).

Data analysis

The colonization density and relative dominance of endophytic fungi isolated from different plant parts were calculated and expressed as percentage (rD %) as described by Fischer and Petrini [22].

$$\%CF = \frac{\text{Number of segments colonized } (N_c)}{\text{Number of segments plated } (N_t)} \text{ X } 100.$$

$$rD\% = \frac{\text{Total number of segments colonized by an endophyte}}{\text{Total number of segments plated}} \text{X } 100.$$

Preparation of endophyte extracts

Pieces of agar (0.5-0.5 cm^2) containing actively growing endophytic fungi were inoculated on to 2000mL Erlenmeyer flasks containing 1000mL of sterile potato dextrose broth. The flasks were incubated under stationary conditions with alternate light (8h) and dark cycles (16h) at 25 ± 2^{o}C for 28days. The mycelial mat submerged in the broth was sonicated. The broth and the mycelial extract were combined, mixed and filtered with four layers of cheese cloth, mixed with equal volumes of different solvents of increasing polarity (hexane>ethyl acetate>chloroform>methanol). The filtrate was evaporated to dryness in a rotary evaporator, concentrated in a vacuum concentrator, stored at -20^{0}C. The dried fractions of the endophytes redissolved in respective solvents were tested for antioxidant and antimicrobial properties.

Biological activities of the endophyte extracts

Determination of antioxidant activity

The ability to scavenge the free radicals by endophytic extracts was determined by DPPH and ABTS radical scavenging assays as follows:

DPPH radical scavenging assay

DPPH (1, 1 –Diphenyl-2-picryl hydrazyl) method for the determination of the antioxidant potential was followed as described by Brand-Williams *et al.* [23]. 300 µM DPPH (Sigma) was prepared in methanol. Different concentrations of the test solutions were prepared (2-10 mg/mL) and 5 µl of the test solutions were taken in 96 well microtiter plate, to which 95µl of DPPH was added. The plate was incubated for 30min in dark at room temperature. The absorbance was read at 517nm using plate reader (Infinite M200Pro). Ascorbic acid (A) and Quercetin (Q) were used as positive control. The results were expressed in terms of total antioxidant capacity (TAC). IC_{50} values were determined and the antioxidant activity was expressed as % DPPH radical scavenging activity, which is calculated as follows:

$$\%\text{DPPH radical scavenging activity} = \frac{(Ac-A)}{Ac} \times 100$$

Ac= absorbance of control
A=absorbance of test sample

ABTS assay

The antioxidant activity was determined by ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) method as described by Re *et al.* [24]. ABTS (Sigma) was diluted to give a solution of 7 mM concentration. For the production of ABTS radical cations, 7mM ABTS and 2.45 mM potassium persulfate was prepared, mixed and incubated in dark at room temperature for 12-16h. The mixture was diluted in methanol to give an absorbance of 0.70 at 734nm. 10µl of the extract of different concentrations were taken, mixed with 990 µl of ABTS and the absorbance was measured at 734nm after 5min. Ascorbic acid (A) and quercetin (Q) were used as positive control. A dose-dependent curve was plotted to calculate the IC_{50} value.

Determination of total phenolics of endophyte extracts

The total phenolic content of endophyte extracts was determined according to Folin-Ciocalteu method according to Singleton *et al.* [25]. 100µl of the endophyte extract was mixed with 500µl of FC reagent (1:1 diluted with distilled water) and was incubated at room temperature for 5min. To this mixture, 1.5mL of the Aqueous Na_2CO_3 was added, mixed by vortexing. The absorbance of the solution was measured at 734 nm using UV/visible light spectrophotometer after incubation at 37^0C for 2h. 2mL of aqueous Na_2CO_3 in 2.5mL of distilled water was taken as blank. Gallic acid was taken as standard and the values were expressed as gallic acid equivalents.

Antimicrobial activity

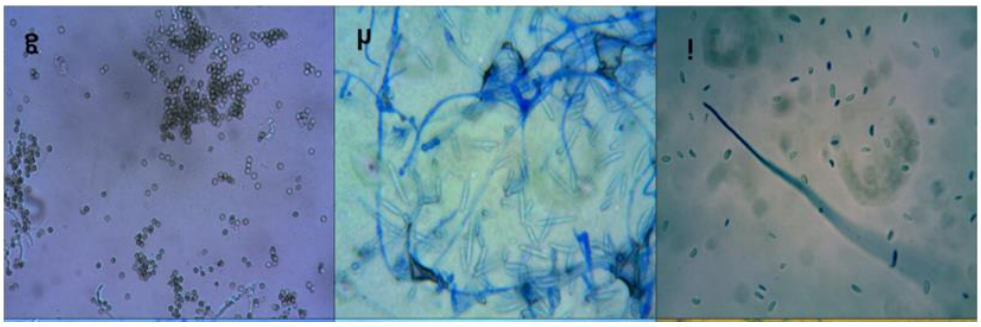
The ability to inhibit visible growth of bacteria by endophytic extracts was assessed by disc diffusion method as decribed by Nostro *et al.* [26]. Endophytic extracts showing good antioxidant potential were selected for the assessment of antimicrobial properties. Extracts of *F. oxysporum*, *A. versicolor*, and *L. theobromae* were prepared to get a concentration of 1mg/mL. Gram-positive bacteria like *Staphylococcus aureus* MTCC 96 and *Bacillus cereus* MTCC 430 and Gram-negative bacteria like *Escherichia coli* MTCC 724 and *Shigella flexnerii* MTCC 1457 were used to assess the antibacterial activity. Prior to the study, test bacteria were cultured in a sterile Muller Hinton broth and incubated overnight at 37^0C. The cultures were centrifuged and the pellets were suspended in sterile water so as to get 10^5-10^6CFU/mL and plated on Muller Hinton agar [27]. About 10-15µL of the endophytic extracts were added to the sterile discs, dried and placed on the culture plates containing bacterial cultures. The plates were incubated at 37 °C for 18 - 24h. Chloramphenicol (1mg/mL) and ethyl acetate were used as positive and negative controls respectively.

Results

Endophytic fungal assemblages of *H. indicus*

Fungal endophytes were isolated from different parts of *H. indicus*. A large and diverse group of endophytic fungi have been isolated from roots when compared to other parts of the plant. This is the first report on the enumeration of endophytic fungi from roots of *H. indicus*.

Endophytic fungi belonging to the species of *Fusarium* like *F. oxysporum*, *F. solani*, *F. decemcellulare*, *F. moniliformae* were found to be the dominant colonizers of the root when compared to other group of fungi. Some of the isolated endophytic fungi expressed tissue specificity and dominance by occurring only in certain tissues of the plant. *Sclerotium rolfsii*, belonging to the class Agaricomycetes was recovered from root samples of *H.indicus* collected from Chamundi hills, but absent in the samples collected from other habitats. It was also found that *Lecanicillium aphanocladii* (*Acremonium aranearum*), is the first report from the roots of *H. indicus* is present only in the samples collected from Gonikoppal regions, which is a part of Western Ghats (Fig. 1).



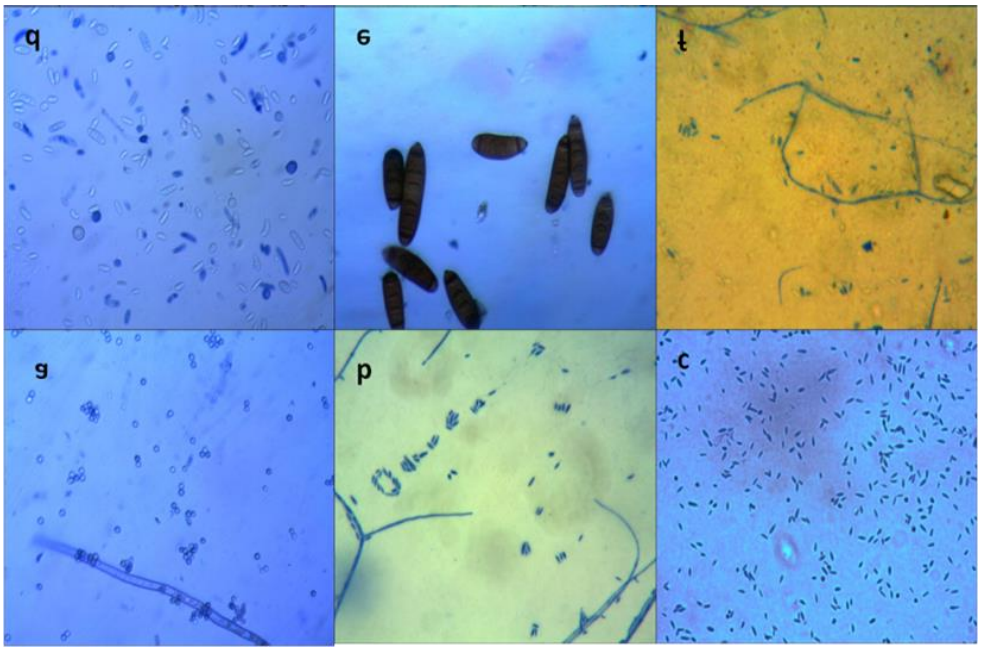


Fig 1: Conidial characteristics of some endophytes isolated from Hemidesmus indicus (a) Cladosporium sphaerospermum (b) Lecanillium aphanocladii (c) F. moniliformae (d) F. oxysporum (e) Bipolaris cynodontis (f) Fusarium spp. (g) Aspergillus sydowii (h) F. decemcellulare (i) C. gleosporioides

The amplified ITS sequences of Endophytes correspond to the band size of 800bp (Fig.2).

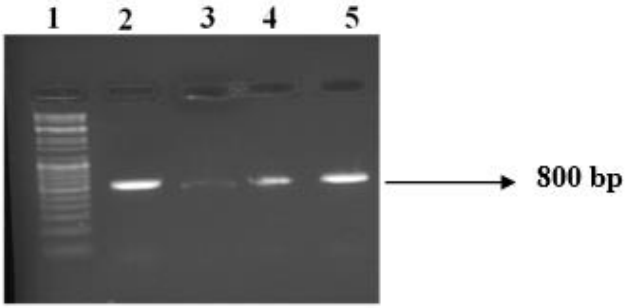


Fig 2: PCR amplifications of ITS regions of selected endophytes Lanes I and 2: *Aspergillus sydowii;* Lane 3: *l'usariuntsp;* Lane 4: *Lecanicilliumaphanockulii*

The sequences compared using BLAST tool showed 90-96% homology with the reference sequences of respective fungi (Table 1).

Table 1: Concentration and purity of the DNA extracted from fungal endophytes of *H. indicus*

Isolate code	Taxa	Conc. of DNA (ng/µL)	Purity of DNA (A_{260}/A_{280})	Percentage similarity to the nearest genera	GenBank accession number
CH19	*Aspergillus sydowii*	832	1.72	92%, *Aspergillus* spp.	MH594212
KH06	*Aspergillus sydowii*	759	1.75	92%, *Aspergillus* spp.	MH594213
KH22	*Fusarium* spp.	1010	1.7	90%, *Fusarium* spp.	MH594214
KH24	*Lecanicillium aphanocladii*	982	1.85	96%,*Lecanicillium aphanocladii*	MH594215

Assesment of relative frequency and colonization of endophytes

Larger number of endophytic fungi was found in root. Nine frequently encountered endophytic fungal groups were found in root, stem and leaves of *H. indicus*. *Fusarium* spp. were scored from root (14.82%), stem (7.16%) and leaves (1.16%). Species of *Fusarium* like *F. solani*, *F. decemcellularae*, *F. moniliformae*, *F.oxysporum*, *F. verticillioides* were found to be colonising the root parts and *F. oxysporum* is a dominant endophyte colonising all parts of the plant **(**Table 2). Other endophytic taxa were concentrated more in roots followed by stem and leaves. The dominant colonizers belonged to the class Hyphomycetes, followed by Euriotiomycetes. Some of the fungi of the class dothideomycetes and coleomycetes like *Colletotrichum* spp. colonise the root. *Trichoderma asperellum* presented a relatively high percentage colonization frequency (20) followed by *Fusarium solani* (19).

Table 2: Occurrence of endophytic fungi in different parts of *H. indicus* collected from three different regions

Endophytic fungi	Gonikoppal			Kirugavalu			Chamundi Hills			Total isolates of each fungus	% CF	Dominance of the fungi
	R	S	L	R	S	L	R	S	L			
Aspergillus sydowii	15	-	-	-	-	-	+	-	10	25	4.16	4.57
Aspergillus versicolor	15	08	-	-	-	-	12	-	-	35	5.83	6.40
Bipolaris cynodontis	12	-	01	-	-	-	-	-	02	15	7.50	8.24
Bipolaris spp	05	01	-	02	-	01	-	01	-	10	5.00	5.49
Cladosporium sphaerospermum	-	-	-	-	-	-	03	-	-	03	1.50	1.64
Colletotrichum dematium	05	-	-	-	-	-	-	-	-	05	2.50	2.74
Colletotrichum gleosporioides	06	-	-	04	-	-	-	-	-	10	5.00	5.49
Curvularia eragrostidis	10	-	-	-	-	-	-	-	-	10	5.00	5.49
Curvularia geniculate	-	-	-	10	-	-	-	-	-	10	1.66	0.85
Curvularia lunata	10	06	-	-	04	-	-	-	-	20	10.0	10.99
Fusarium decemcellularae	06	-	-	-	-	-	-	-	-	06	3.00	3.29
Fusarium moniliformae	07	-	-	04	-	-	04	-	-	15	7.50	8.24
Fusarium oxysporum	18	04	02	15	04	03	20	04	03	73	36.5	40.11
Fusarium solani	-	08	07	05	06	-	04	-	08	38	19.00	20.88
Fusarium spp.	-	-	-	-	02	03	-	-	-	05	2.50	2.74
Fusarium verticilloides	07	-	05	-	-	-	-	-	-	12	6.00	6.59
Lasiodiplodia theobromae	10	-	-	-	-	-	-	-	-	10	5.00	5.49
Lecanicillium aphanocladii	15	-	-	-	-	-	-	-	-	15	7.50	8.24
Phoma spp.	10	-	-	-	-	-	-	-	06	16	8.00	8.79
Phomopsis spp.	07	-	-	05	-	03	-	-	-	15	7.50	8.24
Sarocladium striatum	03	-	-	-	-	-	-	-	-	03	1.50	1.64
Sclerotium rolfsii	-	-	-	-	-	-	16	-	-	16	8.00	8.79
Trichoderma asperellum	28	-	05	07	-	-	-	-	-	40	20.00	21.98
Trichoderma viridae	-	-	-	15	-	05	08	-	-	28	14.00	15.38

Note: R represents total number of isolates recovered from root segments; S represents total number of isolates recovered from stem segments and L represents total number of isolates recovered from leaf parts and - indicates absence of endophytic fungus.

Determination of total phenolics and antioxidant activities of endophytic extracts

The ethyl acetate fractions of *F. oxysporum* and *Aspergillus* spp. exhibited higher concentration of phenolics. The results were expressed as Gallic acid equivalents/mg of the sample. The total phenolic content ranged from 5 to 45mg GAE/mg of the extract.

Ethyl acetate fraction of *F. oxysporum* at the concentration of 50µg/mL exhibited higher DPPH activity of 55.95±3.29% with the IC_{50} value of 45µg/mL. The extracts of other endophytes like *Aspergillus sydowii*, *F. solani* and *B. cynodontis* also showed promising antioxidant activities. In ABTS assay, 50µg of ethyl acetate fractions of *F. oxysporum* showed free radical scavenging activity of 54±3.35%, followed by *A. sydowii* and *F. solani* (Table 3).

Table 3: Antioxidant and total phenolics of different solvent extracts of endophytes isolated from *H. indicus*

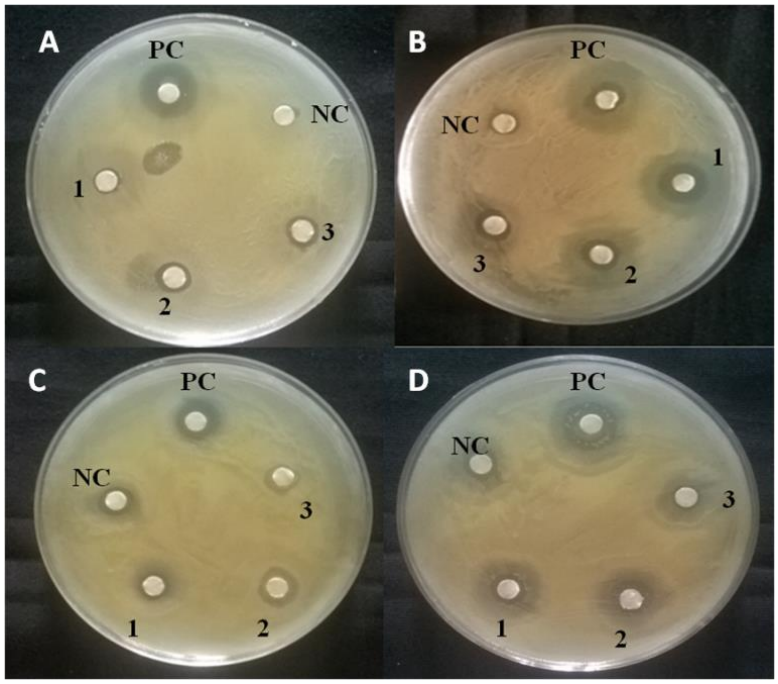
Endophytic extracts/ positive controls	DPPH (IC_{50}) (µg/mL)[a]			ABTS (IC_{50}) (µg/mL)[a]			Total Phenol content (mg/mL)[a]		
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
Fusarium oxysporum	-	44.66±0.57	-	-	45.00±0.20	49.16±0.37	-	45.10±0.36	-
Aspergillus sydowii	47.5±0.5	48.83±0.28	49.46±0.41	-	41.28±0.62	-	-	43.89±0.17	49.43±0.40
Lasiodiplodia theobromae	-	31.66±0.57	48.19±0..28	-	-	-	-	5.10±0.09	-
Bipolaris cynodontis	-	49.29±0.50	-	-	-	-	-	5.16±0.05	-
Fusarium solani	48.33±0.57	49.33±0.28	48.70±0.26	-	42.60±0.52	48.27±0.26	-	42.35±0.56	48.46±0.45
Ascorbic acid	25.16±0.28	24.83±0.28	25.16±0.28	25.60±0.52	25.16±0.28	23.13±0.32			
Quercetin	24.83±0.76	24.16±0.28	23.83±0.76	25.66±0.49	25.16±0.28	25.26±0.64			

*Total phenolic content was expressed as mg gallic acid equivalent/mg of the extract

[a] mean±SD, – represents no activity from endophytic extracts

Antimicrobial activity

Endophytic extracts were selected to screen for their potential to inhibit the growth of pathogenic bacteria (Fig. 3). The extracts were found to be effective against both Gram-positive and Gram-negative bacteria. Overall, the zone of inhibition ranged from 1mm to 1.2cm. The extracts of *F. oxysporum* and *Aspergillus* spp. showed promising antibacterial activity with inhibition zones corresponding to 1.2 cm and 0.8 cm respectively (Table. 4)



(A) Fig. 3: Antibacterial activity of the ethyl acetate fractions of endophytes against Gram positive and Gram negative bacteria, (A) *S.aureus* (B) *B.cereus* (C) *E.coli* (D) *S. flexnerii*, PC- positive control; NC-negative control; 1- *F. oxysporum* extract; 2- *Aspergillus sydowii* extract; 3- *B. cynodontis* extract.

Table 4: Antimicrobial activity of the endophytic ethyl acetate extracts of *H. indicus.*

Test microorganisms	Zone of inhibition (in mm)			
	Fusarium oxysporum	***Aspergillus versicolor***	***Bipolaris cynodontis***	**Chloramphenicol**
Staphylococcus aureus	6.0	3.0	1.0	10.0
Bacillus cereus	12.0	7.0	2.0	15.0
Escherichia coli	3.0	2.0	1.0	9.0
Shigella flexnerii	7.0	5.0	3.0	15.0

Discussion

Large scale exploitation of plants in an unscientific manner has resulted in the depletion of valuable natural resources. Hence, the need for alternative sources with similar bioactive potential as that of host is ever increasing. Endophytes, as sources of bioactive metabolites are proved to be promising as therapeutics.

A large number of diverse endophytes are isolated from roots when compared to other parts of *H. indicus*. However, some leaf endophytic fungi from *H. indicus* like *Alternaria helianthi*, *F. oxysporum*, *F. moniliformae*, *Phomopsis* spp, *Acremonium strictum*, *Aspergillus niger*, *A. flavus*, *Cladosporium* spp, *Trichoderma* spp, *Myrothecium* spp, *Colletotrichum* spp are previously reported [28]. Some of the endophytes are localized to roots while some are present in shoot parts of the plant. Endophytic *A. versicolor* produces a wide range of secondary metabolites. It is a commonly isolated endophytic fungus from *Antidesma madagascariense* [29]. It is also reported as an endophyte of *Taxus baccata* and *T. brevifolia* producing taxane under *in vitro* conditions [30]. Species of *Aspergillus* are effective as biocontrol agents [31]. Also, [32] reported the production of extracellular enzymes like amylase, pectinase and proteinase by *Aspergillus* spp. which is an endophyte of *Moringa olifera* producing antibiotic active substance [33]. *A. fumigatus* isolated from twigs of *Juniperus communis* produces deoxypodophyllotoxin and has antioxidant, antimicrobial activities and biocontrol properties [34, 35]. The fungus isolated from roots of *Pulicaria crispa* is known to produce γ-butyrolactones and Aspernolides L and M known to have antimicrobial, antileishmanial, antimalarial and cytotoxic activities [36]. Thus, presence of the same endophyte in roots of *H. indicus* correlates antimicrobial and antioxidant potential of endophytic fungi.

Some endophytic fungi are known to exhibit host, habitat and tissue specificity [37]. Diverse endophytic fungal communities are isolated in the samples collected from Western Ghats and can be attributed to the specialized ecological niches. The endophytic fungal community and can be further analysed for the production of economically important compounds. Some of the biocontrol strains of *Trichoderma* like *T. asperellum* and *T. viridae* are isolated as endophytes from *H. indicus*. Endophytic *Trichoderma brevicompactum* is known to produce trichodermins which have antifungal activity [38]. The dominant colonizers of *H. indicus* like the genus *Fusarium* is of enormous importance as it produces bioactive metabolites like flavipesins, guignardic acid, ergosterol, α and γ tocopherol, asperfumin, fumigaclavines, festuclavines, tryptostatin B and several others which are useful as antimicrobial agents. Production of solaniol from endophytic *F. solani* JK10 of *Chlorophora regia* [39] is a promising antimicrobial agent. *F. verticillioides* is a common endophyte of maize [40], which is also isolated from fruits of *Flacortia inermis*, synthesizing shikimic acid [41]. *Colletotrichum gleosporioides* is isolated as endophyte during current study. The organism is previously reported as an endophyte of *Piper ornatum*, produces Colletotrialid, having anticancer and antioxidant potential [42].

Identification of endophytes by molecular tools like BLAST helped in assessing the organisms to their species level. Sequences showing various percentages of identity with reference sequences are considered to assess the organisms at species and genus level [43]. High relative frequency in the

plant materials indicated high dispersal and colonization rate of the endophytic fungi. DPPH assay is widely used for the determination of antioxidant potential of plant extracts/endophyte extracts. Free radical scavenging capacity exhibited by dominant endophytes are valuable since it indicates that the active components present in the extract help in eliminating lipid peroxy radicals, hydroxyl radicals and superoxide anion radicals. Further, the ability of the extracts to inhibit the growth of pathogenic bacteria shows that the extracts may be employed as antibacterial agents replacing the use of synthetic drugs, Potent antimicrobial metabolites is reported to be produced from endophytic *Aspergillus fumigatus* of *Quercus variabilis* [44]. Thus, metabolite profiling of such organisms help in exploring several potent compounds having therapeutic applications.

Conclusion

Endophytes are the valuable sources of secondary metabolites with a broad range of biological activities and are applicable as therapeutics. The endophytes of *H. indicus* are found to be diverse. Isolation of root endophytes from the plant and screening for their biological activities helps in enumerating associated fungal communities and identifying potent bioactive metabolites. Further, sequence similarity searching through BLAST helped in identification of endophytes. This is the first report on the isolation of root endophytic fungal communities of *H. indicus.* The dominant endophytes which are reported here are known to produce secondary metabolites which are rich in phenolic compounds and have promising antibacterial and antioxidant activities, which are the future candidates for screening novel bioactive molecules.

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Conflicts of Interest

Authors declare no conflict of interest.

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