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Antibacterial profiling of endophytic fungi sourced from *Justicia betonica*, a medicinal plant from the secluded Western Ghats region

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Abstract

The present study focused on exploring the endophytic fungal diversity associated with an ethnomedicinally valued plant, *Justicia betonica* located in the Western Ghats region of Karnataka and evaluation of their antibacterial potential. A total of 210 endophytic fungal isolates were obtained from healthy leaf and stem tissues. The isolates were grouped into 17 distinct genera, including morphospecies. The *Colletotrichum* is one of the most predominant genera representing 18.1% of the total isolates, followed by *Cladosporium* (14.8%), *Pestalotiopsis* (7.14%), and *Alternaria* (6.7%). The Shannon-wiener diversity (H) showed that fungal diversity in both plant tissues does not have significant differences. The antibacterial potential of the isolates was evaluated using a primary agar plug diffusion technique in which 28 isolates showed prominent antibacterial activity. Nine isolates that showed the highest antibacterial activity were subjected to secondary antibacterial screening using ethyl acetate extracts. The extracts showed varying inhibitory activity against both the pathogens *Escherichia coli* and *Staphylococcus aureus* with inhibition zone ranging from 14 to 32 mm. A *Xylaria* sp. designated as PJJBLF53 displayed the maximum inhibition zone against both the pathogens tested when compared to the standard antibiotic, gentamicin. The study indicates that *J. betonica* harbors diverse endophytic fungi that possess potent antibacterial potential, which could be investigated further for the isolation of novel antibacterial compounds.

Keywords – Agar plug diffusion assay – Antibacterial activity – Diversity indices – Fungal endophytes – *Xylaria* sp.

Introduction

Antibiotics have been playing a significant role in combating infectious diseases caused by microorganisms. However, the excessive and irrational usage of many antibiotics has resulted in the arising of antibiotic-resistant strains (Laxminarayan et al. 2013). Thus, the efficiency of antibiotics has intensely decreased in treating common infectious diseases, and sometimes they are impossible to treat as they become less effective (Neu 1992). Consequently, there is an urgent need for new natural products for antimicrobial drugs against synthetic drugs. Plants have been used to harness novel antimicrobial products (Compean & Ynalvez 2014). In most developing countries, almost

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80% of people rely on medicinal plants to treat infections caused by pathogens (Ekor et al. 2014). However, overexploitation has led to the extinction of several medicinally important plants (Brower 2008). Hence, the interest shifted towards fungal endophytes, which reside inside these plants without causing harm to the host and thus may produce similar compounds found in medicinal plants (Venugopalan & Srivastava 2015). The breakthrough in this area of endophyte bioprospection was the discovery of taxol, also known as paclitaxel, an anticancer compound from the endophytic fungus *Taxomyces andreanae* that inhabits Pacific Yew *Taxus brevifolia* (Strobel et al. 2003). Since then, they have been proven to be a wealthy pool of new bioactive, structurally unique secondary metabolites and have become promising sources of natural drugs (Nisa et al. 2015).

Endophytic fungi colonize the intercellular spaces of healthy tissues of plants without producing any deleterious effect on the host (Strobel et al. 2003, Rodriguez et al. 2008, Doilom et al. 2017). They help plant growth promotion by producing phytohormones (Omomowo & Babalola 2019), increasing tolerance to abiotic/biotic stresses (Leitão & Enguita 2016), and resistance against phytopathogens (Mejía et al. 2008, Hyde et al. 2019, De Silva et al. 2019). Several bioactive compounds with a wide range of bioactivities such as antimicrobial (Kumar et al. 2014), anticancer (Puri et al. 2005), antidiabetic (Singh & Kaur 2016), insecticidal (Daisy et al. 2002), antioxidant (Strobel et al. 2002), anti-inflammatory (Deshmukh et al. 2009), neuroprotective (Lin et al. 2008), antimalarial (Tansuwan et al. 2007), and immunosuppressive (Lee et al. 1995) activities have been isolated from these fungal endophytes.

Justicia betonica L. is commonly called squirrel tail, which is an ethnomedicinal plant used in the treatment of various gastrointestinal ailments. In India, the inflorescence is used to treat vomiting and constipation (Jeruto et al. 2008). A decoction of the whole plant is used to treat stomachaches by people of the Lou tribe in Tanzania (Rao et al. 2006). In Kenya, the flowers and leaves of ash trees are used as medicines for cough, while an infusion of leaves is used for snake-bite (Kokwaro & Johns 1998). The poultice made from the leaves is used to treat boils in Ceylon, and swellings in Malaya (Jeruto et al. 2008). Even though many species of Justicia have been screened for their potential fungal endophytes, *J. betonica* was left underexplored even with its ethnomedicinal importance. In the present study, we explored the bioactive potential of the fungal endophytic communities associated with *J. betonica* located in one of the biodiversity hotspot, the Western Ghats of India.

Materials & Methods

Collection of plant

The fresh, healthy plant materials were collected from the Virajpet taluk of Kodagu district of the Western Ghats region of Karnataka, India (12°13’30” N, 76°00’41” E) in sterile polythene bags. The plant materials were bought to the laboratory and processed immediately for the isolation of endophytic fungi.

Isolation of endophytic fungi

The plant material was washed initially to remove soil and dust particles with running tap water. After shade drying, the stems and leaves were cut into small segments (0.5 cm^2) using a sterile scalpel. These segments were subjected to surface sterilization by washing in 70% ethanol (C_2H_5OH) for 1 min, followed by 4% sodium hypochlorite (NaOCl) for 4 min and washing thrice in sterile distilled water. The excess moisture was blotted. Surface sterilized stem and leaf segments were placed equidistantly on the surface of water agar plates (10 segments/plate), previously amended with 100 mg L^{-1} of chloramphenicol to prevent contamination by bacteria. The plates were incubated at 25 ± 2°C for 3 to 4 days and monitored daily for the fungal growth from each segment. Fungal hyphae emerging from the leaf and stem segments were transferred onto fresh potato dextrose agar (PDA) plates and incubated at room temperature (25 ± 2°C) for about 5–7 days to establish pure cultures (Rakshith et al. 2013).

Identification of endophytic fungi

The endophytic fungi were identified based on a thorough examination of macro and micromorphological characteristics up to the generic level by referring to standard fungal identification manuals (Ainsworth et al. 1973, Barnett & Hunter 1998). The isolates were categorized according to their genus and isolates that failed to sporulate were designated as unidentified morphospecies.

Data analysis

The endophytic fungal diversity of *J. betonica* was quantitatively analyzed in terms of colonization frequency (CF), isolation rate (IR), species richness (S), total abundance, evenness along with Shannon-Wiener (H) and Simpson (1-D) diversities. The frequency of colonization was expressed as the number of leaf/stem fragments from which one or more endophytic fungi were recovered divided by the total number of leaf/stem segments plated × 100 (Kumar & Hyde 2004). The total number of fungal endophytes recovered from the leaf/stem segments divided by the total number of leaf/stem segments plated × 100 was expressed as isolation rate (IR) (Arora et al. 2019, Kumar & Hyde 2004). The relative abundance is described as the total counted number of fungal isolates of each species divided by the total number of all species of isolates obtained. Species richness is the total number of fungal species obtained from each leaf and stem (Arora et al. 2019), and the species evenness is the relative contribution of each species to the total biomass (Polley et al. 2003). Species richness, total abundance, evenness, Shannon-Wiener diversity index, and Simpson diversity index were calculated using an online diversity calculator (http://www.changbioscience.com/genetics/shannon.html).

Antibacterial activity

Agar plug diffusion assay

The isolated endophytic fungi were subjected to preliminary antibacterial screening through the agar plug diffusion method. This permitted a rapid and qualitative identification of the bioactive isolates. Mycelial agar plugs approximately 6 mm in diameter of an actively growing fungal endophyte culture were cut with a flamed cork borer and transferred to the surface of Mueller Hinton agar (MHA) plates, previously seeded with test microorganisms i.e., *Escherichia coli* and *Staphylococcus aureus*. The plates were kept in a refrigerator at 4°C for 10 min for the diffusion of metabolites. Further, these plates were incubated at 37°C for 24 hours and observed for the zone of inhibition around the agar plugs (Nuthan et al. 2020a).

Preparation of fungal crude extracts

The selected fungal strains were inoculated into a 1000 ml conical flask, containing 300 ml of potato dextrose broth (PDB) and incubated at 25 ± 2°C for 45 days. After incubation, the mycelial mat was separated from the culture broth by filtering through a double-layered cheesecloth. Then, the culture filtrate was transferred to a 1000 ml bottle, and an equal volume of ethyl acetate was added. The contents of the bottle were agitated vigorously for about 20–25 minutes, and transferred to a separating funnel, and left for few minutes till the separation of two distinct phases. The upper organic phase (ethyl acetate), which contained secondary metabolites, was carefully collected in a separate brown bottle, and the process was repeated two times. Finally, the collected organic phase was concentrated in a rotary evaporator under low pressure at 35–40°C (Phongpaichit et al. 2006).

Antibacterial screening by agar well diffusion method

Concentrated ethyl acetate extracts of selected endophytic fungi were subjected for the antibacterial activity assay by agar well diffusion method against bacteria: *S. aureus* (Gram-positive), and *E. coli* (Gram-negative). The bacterial cultures were adjusted to 0.5 McFarland standards, and 100 µL was spread onto the MHA plate. The wells were made using a 6 mm diameter cork borer, and 50 µL of ethyl acetate extract of each strain was added into a separate

well. Gentamicin and ethyl acetate was used as positive and negative controls, respectively. The plates were kept at 4ºC for about 10 min, thus allowing the diffusion of metabolites from the extracts. The plates were then incubated at 37°C for 24 hours and observed for the inhibition zone surrounding the wells (Nuthan et al. 2020b).

Statistical analysis

Agar well diffusion assay was conducted in triplicates, and statistical analyses were carried out using the IBM SPSS software (version 25). The statistically significant differences in the results obtained were calculated using one-way analysis of variance (ANOVA) at $p \leq 0.001$ followed by Tukey's post hoc test with $p \leq 0.05$; the final values were represented as mean ± standard deviation (SD).

Results

Isolation and identification of endophytic fungi

A total of 210 culturable endophytic fungal isolates were obtained from 400 tissue segments (200 each of leaves and stems) of *J. betonica*. The overall procedure is summarized pictorially in Fig. 1, we isolated 117 isolates from leaves and 93 from stems. These isolates were further carefully grouped into 17 genera, including morphospecies based on their macro and micromorphological characteristics. Colony morphology and microscopic images of isolated fungal endophytes are shown in Fig. 2. Most of the isolates were from the class Sordariomycetes (62%), followed by Dothideomycetes (26%) and Agaricomycetes (3%). *Colletotrichum* was the most dominant genus among the endophytic fungal community representing 18.09% of the total isolates, followed by *Cladosporium* with 10.69%. On the other hand, the lowest dominance was observed with *Botryosphaeria* (0.95%). The distribution and colonization frequency (CF%) of endophytic fungi are summarized in Table 1.

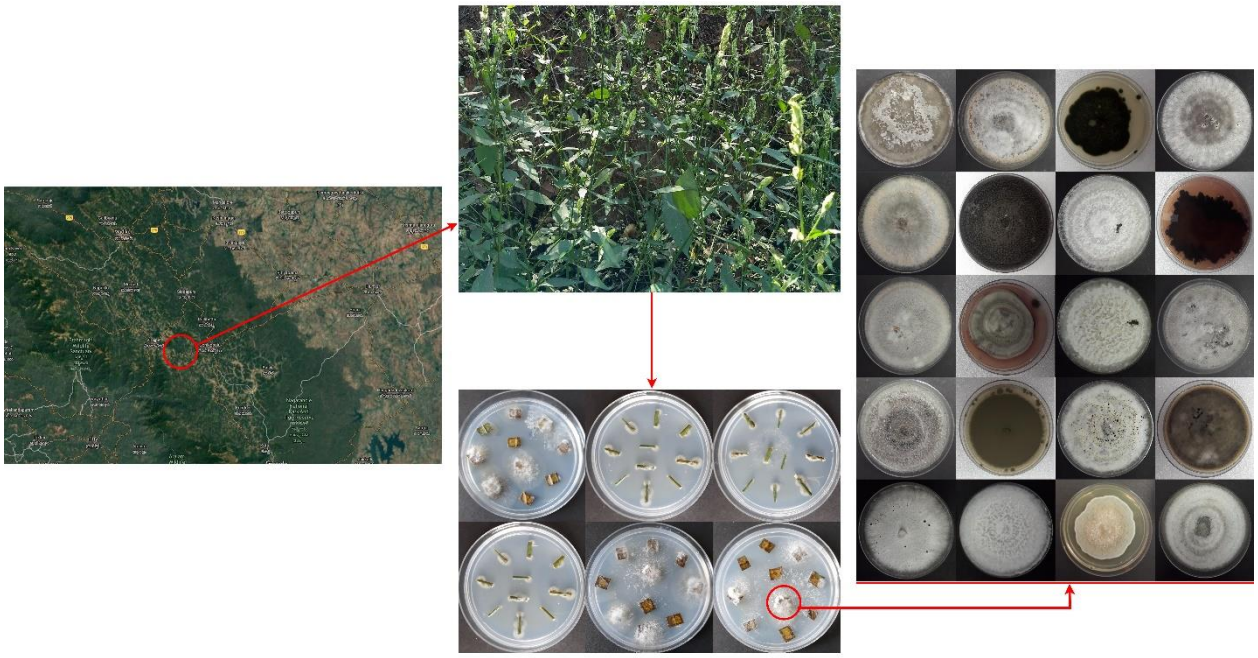

Fig. 1 – Pictorial description of the overall procedure followed for isolation of endophytic fungi from *J. betonica*.

Table 1 Distribution and colonization frequency (CF%) of endophytic fungi from *J. betonica.*

Sl. No.	Class	Family	Fungi	Leaves	Stems	Total	CF%
1	Agaricomycetes	Ceratobasidiaceae	*Rhizoctonia* sp.	3	3	6	1.5
2	Dothideomycetes	Cladosporiaceae	*Cladosporium* sp.	10	21	31	7.75
		Botryosphaeriaceae	*Botryosphaeria* sp.	0	2	2	0.5
		Didymellaceae	*Phoma* sp.	5	3	8	2
		Pleosporaceae	*Alternaria* sp.	9	5	14	3.5
			Curvularia sp.	3	0	3	0.75
3	Sordariomycetes	Sporocadaceae	*Pestalotiopsis* sp.	10	5	15	3.75
		Chaetomiaceae	*Chaetomium* sp.	6	4	10	2.5
		Ceratocystidaceae	*Ceratocystis* sp.	7	5	12	3
		Glomerellaceae	*Colletotrichum* sp.	25	13	38	9.5
		Hypocreaceae	*Gliocladium* sp.	5	4	9	2.25
		Nectriaceae	*Fusarium* sp.	5	5	10	2.5
			Gliocladiopsis sp.	0	3	3	0.75
		Stachybotryaceae	*Myrothecium* sp.	6	7	13	3.25
		Diaporthaceae	*Diaporthe* sp.	3	2	5	1.25
		Xylariaceae	*Xylaria* sp.	7	5	12	3
4	Unrecognized	Mycelia sterilia	Morpho sp.	13	6	19	4.75
			Total	**117**	**93**	**210**	**52.5**

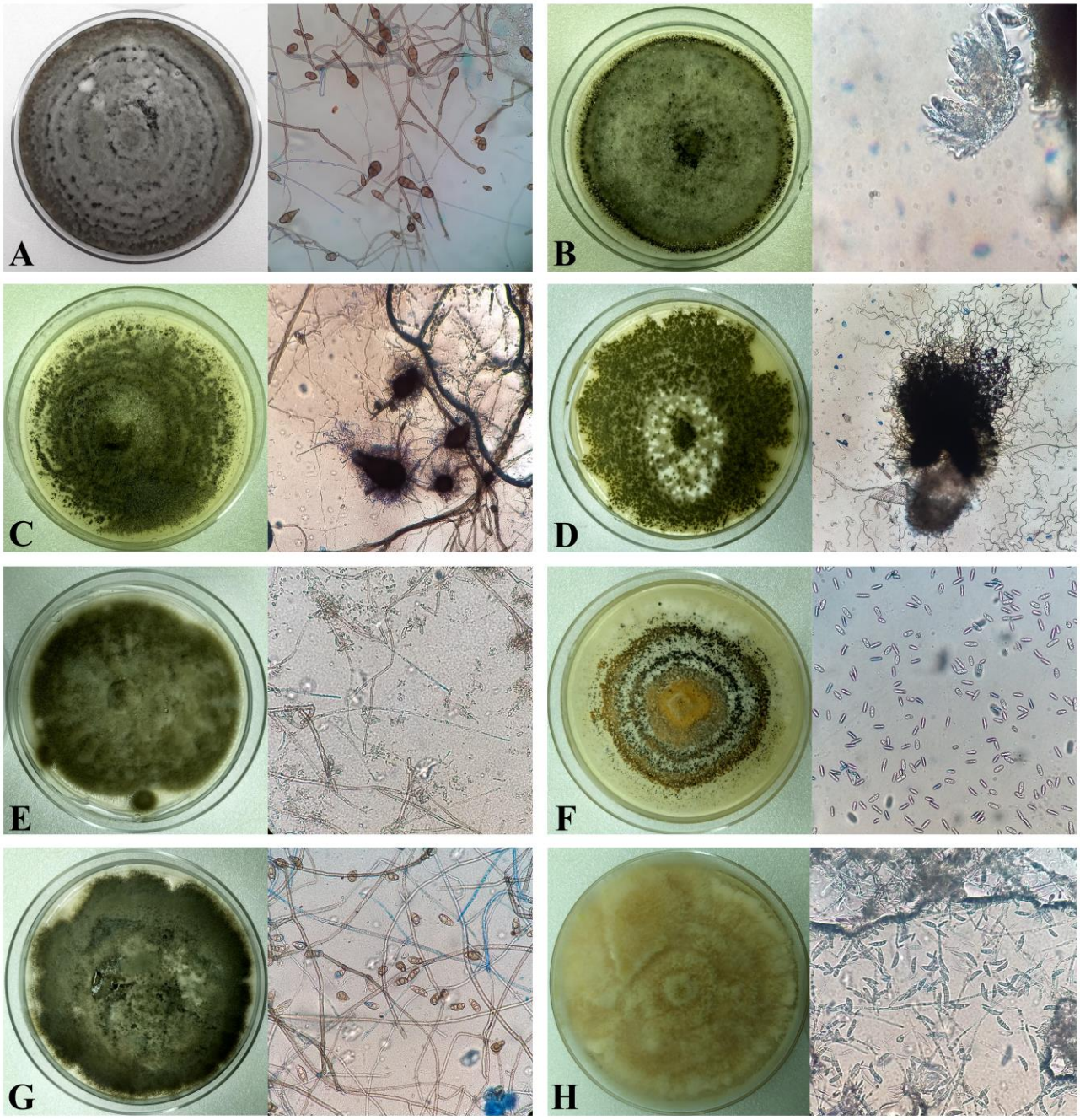

Fig. 2 – Colony morphology and microscopic characteristics of the isolated fungal endophytes from *J. betonica*. A *Alternaria* sp. B *Botryosphaeria* sp. C *Ceratocystis* sp. D *Chetomium* sp. E *Cladosporium* sp. F *Colletotrichum* sp. G *Curvularia* sp. H *Fusarium* sp. I *Gliocladiopsis* sp. J *Gliocladium* sp. K *Myrothecium* sp. L *Pestalotiopsis* sp. M *Phoma* sp. N *Diaporthe* sp. O *Rhizoctonia* sp., (P-S) *Xylaria* sp., and (T, U) Morpho sp.

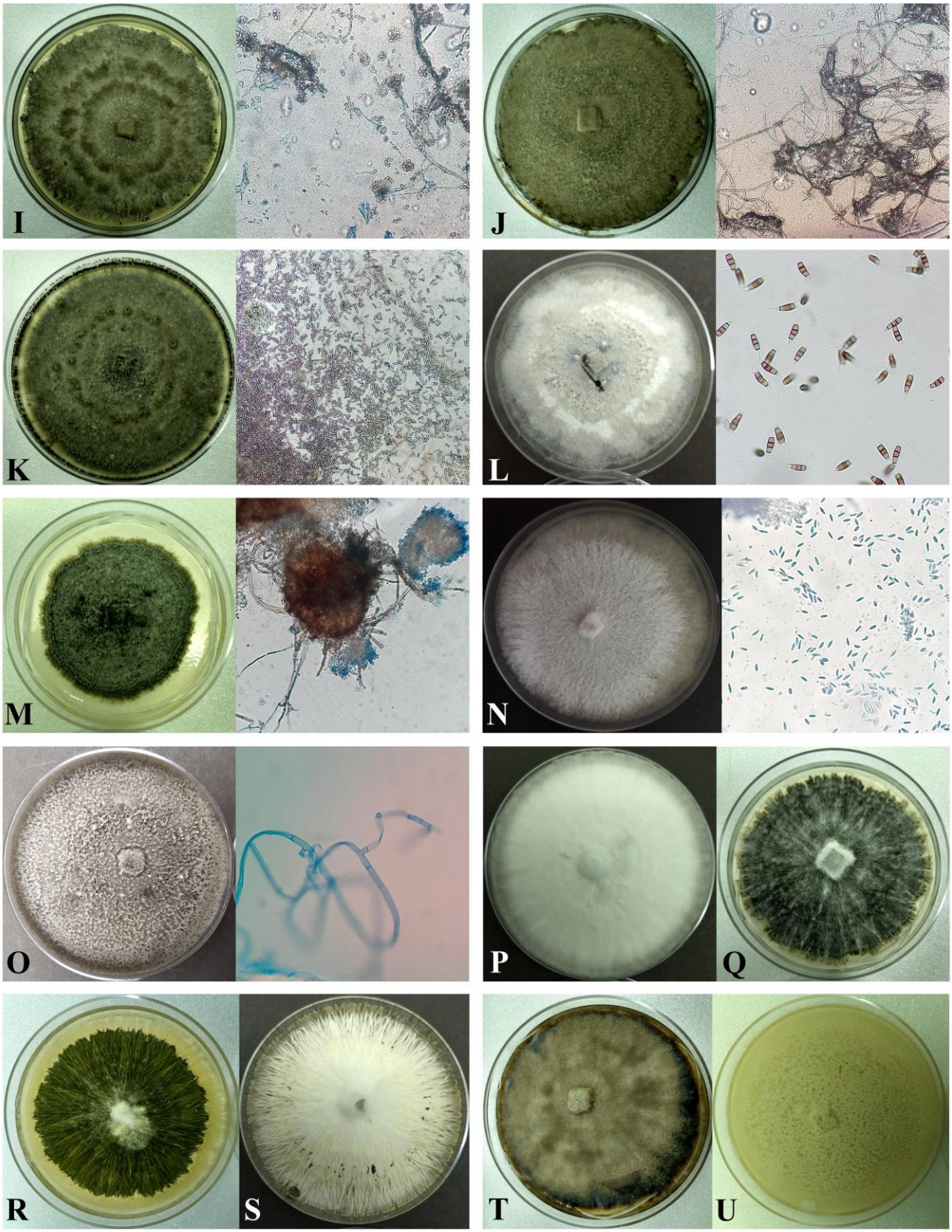

Fig. 2 – Continued.

Data analysis

The colonisation frequency of fungal endophytes was more in leaf (90%) tissues compared to that of the stem parts (81.5%). The isolation rates for leaves and stems were 58.5 and 46.5%,

respectively (Table 2). Both leaves (2.6) and stems (2.5) had a similar Shannon-wiener diversity index (H). The tissue-specific fungal dominance was higher in the leaves when compared to the stems. Highest Species Richness (S) was observed in the stems (17), followed by leaves. Species evenness was slightly lower in leaves (0.9) than in stems (0.2). Diversity indices of endophytic fungi isolated are given in Table 3.

Table 2 Isolation Rate and Colonisation Frequency.

	Leaf	**Stem**	**Total**
No. of Segments	200	200	400
No. of segments yielding endophytic fungi	180	163	343
No. of isolates	117	93	210
Isolation rate (%)	58.5	46.5	52.5
Colonisation frequency (%)	90	81.5	85.75

Table 3 Diversity of endophytic fungi isolated from *J. betonica.*

	Leaf	**Stem**
Shannon-Wiener diversity (H)	2.58	2.47
Species richness (S)	16.0	17.0
Total abundance	234	186
Simpson diversity (1-D)	0.90	0.89
Evenness	0.93	0.90

Antibacterial activity

Agar plug diffusion assay

Among all the tested endophytic fungal isolates, 28 showed prominent antibacterial activity against both test bacteria, *S. aureus* and *E. coli*. The varying range of inhibition zone displayed by individual isolates around the agar plugs is shown in Table 4.

Table 4 Range of inhibition zone of endophytic fungal isolates from *J. betonica* against pathogenic bacteria.

Fungi	**Isolate code**	**Primary antibacterial activity**	
		E. coli	***S. aureus***
Alternaria sp.	PJJBST37	++	+
Alternaria sp.	PJJBLF84	++	-
Cladosporium sp.	PJJBST15	++	-
Cladosporium sp.	PJJBST16	+	+++
Curvularia sp.	PJJBLF117	+	++
Curvularia sp.	PJJBST177	-	++
Fusarium sp.	PJJBLF12	+++	++
Fusarium sp.	PJJBLF41	+	+++
Fusarium sp.	PJJBLF82	+++	++
Fusarium sp.	PJJBLF133	++	+
Morpho sp.	PJJBLF23	+++	++
Morpho sp.	PJJBLF176	+	-
Morpho sp.	PJJBST190	++	+
Morpho sp.	PJJBST193	++	+++

Table 4 Continued.

Fungi	Isolate code	Primary antibacterial activity	
		E. coli	***S. aureus***
Pestalotiopsis sp.	PJJBST54	++	++
Pestalotiopsis sp.	PJJBST188	+	-
Pestalotiopsis sp.	PJJBST191	++	-
Phoma sp.	PJJBLF135	+	++
Phoma sp.	PJJBST209	+++	+
Diaporthe sp.	PJJBLF161	++	+++
Diaporthe sp.	PJJBST187	+	++
Diaporthe sp.	PJJBST199	+	++
***Xylaria* sp.**	**PJJBLF53***	+++	+++
Xylaria sp.	PJJBST111	+	+++
Xylaria sp.	PJJBST179	+	+++
Xylaria sp.	PJJBST185	++	+
Xylaria sp.	PJJBST189	++	++
Xylaria sp.	PJJBLF203	++	++

+: Inhibition zone < 10 mm
++: Inhibition zone between 10–20 mm
+++: Inhibition zone > 20 mm
-: No inhibition zone
***: Isolate with most significant antibacterial activity**

Antibacterial screening by agar well diffusion method.

Eight of nine tested fungal extracts displayed antibacterial activity against both *S. aureus* and *E. coli* with inhibition zone ranging from 13 to 31 mm. Extract of PJJBST53, a *Xylaria* sp. showed the largest zone of inhibition against both *E. coli* (31 ± 1 mm) and *S. aureus* (28.7 ± 0.6 mm), compared with the standard positive control of 21.3 ± 0.6 mm (*E. coli*) and 24.3 ± 0.6 nm (*S. aureus*). The extract of PJJBST209 was inactive against both test organisms. The range and formation of inhibition zones (Mean ± SD) of ethyl acetate extract of endophytic fungi are shown in Table 5 and Fig. 3.

Table 5 Antibacterial activity of crude fungal extracts of selected endophytic fungal isolates from *J. betonica*.

Fungi	Isolate code	Antibacterial activity against (mm)	
		E. coli	***S. aureus***
Fusarium sp.	PJJBLF12	29.00 ± 1.00^{a}	21.00 ± 1.00^{c}
Morpho sp.	PJJBLF23	22.00 ± 1.00^{bcd}	15.00 ± 1.00^{e}
Xylaria sp.	PJJBLF53	31.00 ± 1.00^{a}	28.66 ± 0.57^{a}
Pestalotiopsis sp.	PJJBST54	14.66 ± 0.57^{e}	-NA-
Fusarium sp.	PJJBLF82	24.33 ± 1.52^{b}	13.33 ± 0.57^{e}
Diaporthe sp.	PJJBLF161	23.00 ± 1.00^{bc}	25.00 ± 1.00^{b}
Morpho sp.	PJJBST193	19.33 ± 1.52^{d}	18.66 ± 0.57^{d}
Xylaria sp.	PJJBLF203	16.33 ± 0.57^{e}	14.00 ± 1.00^{e}
Phoma sp.	PJJBST209	-NA-	-NA-
Positive Control	Gentamicin	21.33 ± 0.57^{cd}	24.33 ± 0.57^{b}
Negative Control	Ethyl acetate	-NA-	-NA-

Values given are means of triplicates ± standard deviation of the mean (SDM; significant $p < 0.001$) by one-way ANOVA. Values followed by the same superscript letter(s) within columns are significantly different at $p < 0.05$ by Tukey's post hoc test; NA: No activity

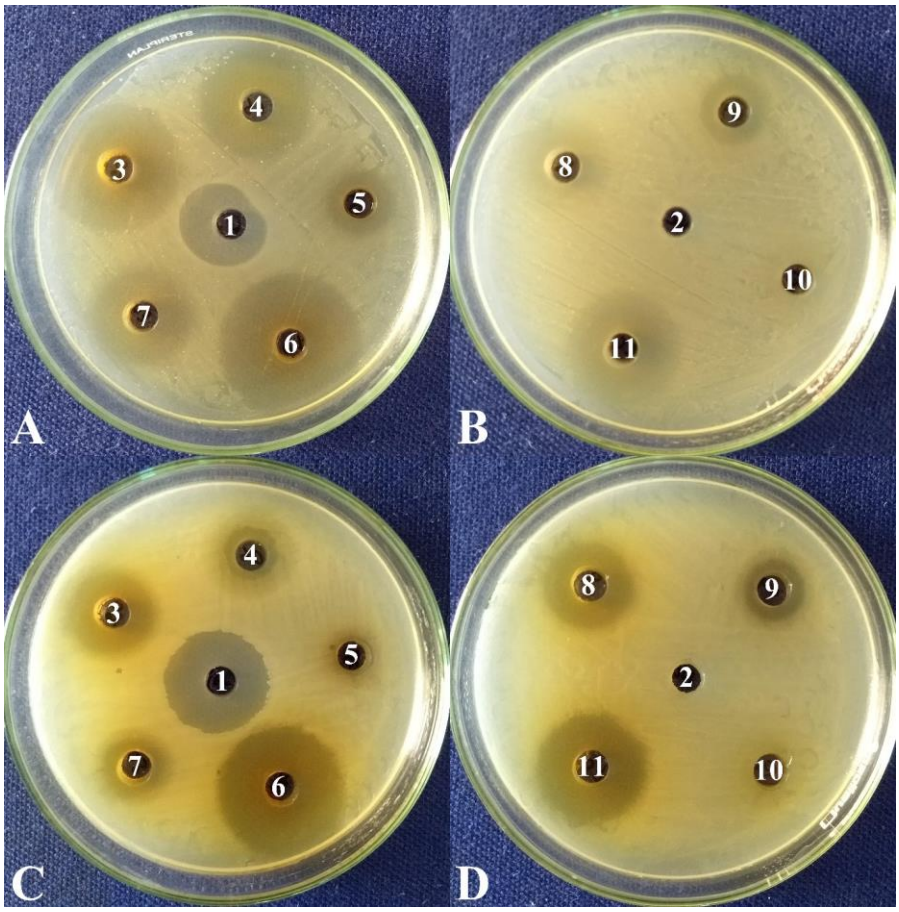

Fig. 3 – Zone of inhibition of selected fungal crude extracts against *E. coli* (A, B) and *S. aureus* (C, D) by agar well diffusion method. 1 – Positive control (Gentamicin), 2 – Negative control (Ethyl acetate), 50 µl Ethyl acetate extracts of isolates 3 – PJJBLF12, 4 – PJJBLF23, 5 – PJJBLF53, 6 – PJJBST54, 7 – PJJBLF82, 8 – PJJBLF161, 9 – PJJBST193, 10 – PJJBLF203, and 11 – PJJBST209.

Discussion

Drug resistance among microorganisms is a major risk to human health, and thus the development of novel antimicrobial compounds is one of the best solutions to mitigate this problem (Laxminarayan et al. 2013). Endophytes from plants with an ethnomedicinal background that is used by native inhabitants for treating several diseases have proven to be the best source for bioactive compounds (Sunaryanto & Mahsunah 2014, Sridhar 2019). Because of this hypothesis, the medicinal plant *J. betonica* used for the treatment of various gastrointestinal complaints was selected for the present study. A total of 210 culturable mycoendophytes were retrieved from leaf and stem tissue segments of *J. betonica.* Among them, 117 isolates were obtained from leaf segments and 93 from stem segments with an isolation rate of 58.5 and 46.5%, respectively. Endophytic fungi were isolated with a similar isolation rate from *Tripterygium wilfordii* by Kumar & Hyde (2004). The colonization frequency of fungal endophytes was more in leaf tissues (90%) as compared to stem parts (81.5%) of the plant and also justifies that foliar parts tend to harbor more mycoendophytic communities when compared to the stem parts (Nuthan et al. 2020a). These tissues represent two distinct micro-environments, subsequently influencing their mycoendophytic communities differently.

Shannon-wiener diversity index (H) and Simpson's diversity index (1-D) were used to determine the diversity of endophytic fungi inhabiting leaves and stems. Leaves showed a higher Shannon-wiener index (2.6) when compared to stems (2.5), similar to the results obtained by Raviraja (2005). The value of Simpson's diversity index lies between 0 and 1, and a high index value indicates higher species diversity. Leaves showed a higher Simpson's index value, indicating a greater species diversity for that particular species, while stems showed higher species diversity in

the study conducted by Du et al. (2020). The measure of evenness is indicated by the relative abundance of species, which was also higher in leaves. Thus, the endophytic fungi of leaves showed more diversity compared to the endophytic fungi of the stems.

The isolates were grouped into 17 genera based on macro and micromorphological characters. Although this type of identification is presumptive, standard manuals were used for the same. Most of the isolates were from class Sordariomycetes (62%), one of the largest classes in Ascomycota. They comprise a highly diverse range of fungi, including endophytes. Sordariomycetes were followed by Dothideomycetes (26%), Mycelia sterilia (9%), and Agaricomycetes (3%). The *Colletotrichum* was the dominant foliar endophytic community isolated whereas *Cladosporium* was the dominant endophytic community isolated from the stems. Thus, tissue specificity was evident among the endophytes of the host. The most recurrent endophytic species isolated were *Colletotrichum* spp., *Cladosporium* spp., *Fusarium* spp., and *Pestalotiopsis* spp. *Colletotrichum* is widely found as an endophytic fungal community in plants. In literature, different species of the endophytic *Colletotrichum* were isolated from many plants such as *Artemisia annua* (Lu et al. 2000), *Justicia gendarussa* (Gangadevi & Muthumary 2008), *Mangifera indica* (Vieira et al. 2014), *Rhizophora apiculate* (Doilom et al. 2017) and *Pandanus* sp. (Tibpromma et al, 2018). *Colletotrichum gloeosporioides* with antimicrobial activity was obtained from *Sonneratia apetala* (Nurunnabi et al. 2018).

In the preliminary screening of endophytic fungi for antibacterial activity through the agar plug diffusion method, 28 isolates showed prominent inhibition zones around agar plugs. Among them, nine isolates with higher antibacterial activity against both the test pathogens were selected for further studies. Ethyl acetate extracts of selected endophytic fungi were tested for their antibacterial efficacy against *S. aureus* and *E. coli* using the agar well diffusion method. Ethyl acetate is used extensively as a solvent for secondary metabolites extraction from fungal culture broth with the added advantage of isolating both types of polar and non-polar metabolites (Bhardwaj et al. 2015). However, it did not influence the antibacterial ability of the fungal extracts as it was subsequently concentrated in a rotary flash evaporator under reduced pressure and moderate temperature. All the tested metabolite concentrates displayed substantial antibacterial activity in the secondary screening assay except *Xylaria* sp. PJJBST209, which lost its bioactivity after metabolites separation. The Ethyl acetate extracts of endophytic *Xylaria* sp. PJJBLF53 displayed the most significant antibacterial activity against both *E. coli* and *S. aureus* with an inhibition zone of 31 ± 1 mm and 28.7 ± 0.6 mm, respectively. Endophytic Xylariaceae members are gaining importance in the natural product drug discovery with the identification of a wide range of bioactive metabolites. The antimicrobial compound Xylabisboein A was obtained from a *Xylaria* sp. isolated from *Bisboecklera microcephala* by Sorres et al. (2015). Cytochalasin H obtained from *Xylaria* sp. isolated from *Annona squamosa* showed cytotoxic effect against HEK 293T cell line (Li et al. 2012). Xylaranol B an active compound obtained from the endophytic fungus *X. papulis* displayed strong phytotoxicity on radish seeds (Amand et al. 2012). The extract *Xylaria* sp. PJJBLF53 showed significant antibacterial activity as compared to the standard antibiotic. Thus, the purification and isolation of bioactive metabolites could pave the way to the isolation of the novel antibacterial drug lead.

Conclusion

The present study has shown that *Justicia betonica* from the biodiversity-rich area is a prominent host for the unique and diversified bioactive mycoendophytic communities. Leaves were more diversified in terms of colonization when compared to stems and dominated by the Sordariomycetes isolates. The preliminary screening of the antibacterial activities indicated that the presence of several isolates from different genera has potent antibacterial properties. The secondary screening proved that extract of *Xylaria* sp. (PJJBLF53) has a significant antibacterial activity, which correlates with the results of agar plug diffusion assay and could be used as a potential source of antibacterial drugs. Thus, further research is intended for the purification and

identification of bioactive compounds from *Xylaria* sp. PJJBLF53 with medicinal properties, leading to the discovery of novel antibacterial drugs that can benefit society.

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References

Ainsworth GC, Sussman AS, Sparrow FK. 1973 – The Fungi: An Advanced Treatise. A Taxonomic Review with Keys: Ascomycetes and Fungi Imperfecti. Volume 4A., Academic Press.

Amand S, Langenfeld A, Blond A, Dupont J et al. 2012 – Guaiane sesquiterpenes from *Biscogniauxia nummularia* featuring potent antigerminative activity. Journal of natural products 75, 798–801.

Arora P, Wani ZA, Ahmad T, Sultan P et al. 2019 – Community structure, spatial distribution, diversity and functional characterization of culturable endophytic fungi associated with *Glycyrrhiza glabra* L. Fungal biology 123, 373–383.

Barnett HL, Hunter BB. 1998 – Illustrated genera of imperfect fungi, 4th ed., The American Phytopathological Society.

Bhardwaj A, Sharma D, Jadon N, Agrawal PK. 2015 – Antimicrobial and phytochemical screening of endophytic fungi isolated from spikes of *Pinus roxburghii*. Archives of Clinical Microbiology 6, 1–9.

Brower V. 2008 – Back to nature: extinction of medicinal plants threatens drug discovery.

Compean KL, Ynalvez RA. 2014 – Antimicrobial activity of plant secondary metabolites: A review. Research Journal of Medicinal Plants 8, 204–213.

Daisy BH, Strobel GA, Castillo U, Ezra D et al. 2002 – Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. Microbiology 148, 3737–3741.

Deshmukh SK, Mishra PD, Kulkarni-Almeida A, Verekar S et al. 2009 – Anti-inflammatory and anticancer activity of ergoflavin isolated from an endophytic fungus. Chemistry & biodiversity 6, 784–789.

De Silva NI, Brooks S, Lumyong S, Hyde KD. 2019 – Use of endophytes as biocontrol agents. Fungal Biology Reviews 33, 133–148.

Doilom M, Manawasinghe IS, Jeewon R, Jayawardena RS et al. 2017 – Can ITS sequence data identify fungal endophytes from cultures? A case study from Rhizophora apiculate 8, 1869–1892.

Du W, Yao Z, Li J, Sun C et al. 2020 – Diversity and antimicrobial activity of endophytic fungi isolated from *Securinega suffruticosa* in the Yellow River Delta. PloS one 15, e0229589.

Ekor M. 2014 – The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in pharmacology 4, 177.

Gangadevi V, Muthumary J. 2008 – Isolation of *Colletotrichum gloeosporioides*, a novel endophytic taxol-producing fungus from the leaves of a medicinal plant, *Justicia gendarussa*. Mycologia balcanica 5, 1–4.

Hyde KD, Xu J, Rapior S, Jeewon R, et al. 2019 – The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Diversity 97, 1–36.

Jeruto P, Lukhoba CL, Ouma G, Otieno D, Mutai C. 2008 – Herbal treatments in Aldai and Kaptumo divisions in Nandi district, Rift valley province, Kenya. African Journal of Traditional, Complementary and Alternative Medicines 5, 103–105.

Kokwaro JO, Johns T. 1998 – Luo biological dictionary. East African Publishers.

Kumar DSS, Hyde KD. 2004 – Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii*. Fungal Diversity 17, 69–90.
Kumar S, Aharwal RP, Shukla H, Rajak RC, Sandhu SS. 2014 – Endophytic fungi: as a source of antimicrobials bioactive compounds. World Journal of Pharmaceutical Sciences 3, 1179–1197.
Laxminarayan R, Duse A, Wattal C, Zaidi AK et al. 2013 – Antibiotic resistance—the need for global solutions. The Lancet infectious diseases 13, 1057–1098.
Leitão AL, Enguita FJ. 2016 – Gibberellins in *Penicillium* strains: challenges for endophyte-plant host interactions under salinity stress. Microbiological research 183, 8–18.
Lee JC, Lobkovsky E, Pliam NB, Strobel G, Clardy J. 1995 – Subglutinols A and B: immunosuppressive compounds from the endophytic fungus *Fusarium subglutinans*. The Journal of Organic Chemistry 60, 7076–7077.
Li Y, Lu C, Huang Y, Li Y, Shen Y. 2012 – Cytochalasin H2, a new cytochalasin, isolated from the endophytic fungus *Xylaria* sp. A23. Records of natural products 6, 121–126.
Lin Z, Wen J, Zhu T, Fang Y et al. 2008 – Chrysogenamide A from an endophytic fungus associated with *Cistanche deserticola* and its neuroprotective effect on SH-SY5Y cells. The Journal of antibiotics 61, 81–85.
Lu H, Zou WX, Meng JC, Hu J, Tan RX. 2000, New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. Plant science 151, 67–73.
Mejía LC, Rojas EI, Maynard Z, Van Bael S et al. 2008 – Endophytic fungi as biocontrol agents of *Theobroma cacao* pathogens. Biological Control 46, 4–14.
Neu HC. 1992 – The crisis in antibiotic resistance. Science 257, 1064–1073.
Nisa H, Kamili AN, Nawchoo IA, Shafi S. et al. 2015 – Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: a review. Microbial pathogenesis 82, 50–59.
Nurunnabi TR, Al-Majmaie S, Nakouti I, Nahar L et al. 2018 – Antimicrobial activity of kojic acid from endophytic fungus *Colletotrichum gloeosporioides* isolated from *Sonneratia apetala*, a mangrove plant of the Sundarbans. Asian Pacific Journal of Tropical Medicine 11, 350–354.
Nuthan BR, Rakshith D, Marulasiddaswamy KM, Rao HY. et al. 2020a – Application of optimized and validated agar overlay TLC–bioautography assay for detecting the antimicrobial metabolites of pharmaceutical interest. Journal of chromatographic science 58, 737–746.
Nuthan BR, Rakshith D, Marulasiddaswamy KM, KM Ramesha et al. 2020b – Mycoendophytic diversity and their antimicrobial potential from two epiphytic orchids of the Western Ghats forests of India. Studies in Fungi 5, 113–124
Omomowo OI, Babalola OO. 2019 – Bacterial and fungal endophytes: tiny giants with immense beneficial potential for plant growth and sustainable agricultural productivity. Microorganisms 7, 481.
Phongpaichit S, Rungjindamai N, Rukachaisirikul V, Sakayaroj J. 2006 – Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia* species. FEMS Immunology and Medical Microbiology 48, 367–372.
Polley HW, Wilsey BJ, Derner JD. 2003 – Do species evenness and plant density influence the magnitude of selection and complementarity effects in annual plant species mixtures?. Ecology Letters 6, 248–256.
Puri SC, Verma V, Amna T, Qazi GN, Spiteller M. 2005 – An endophytic fungus from *Nothapodytes foetida* that produces Camptothecin. Journal of natural products 68, 1717–1719.
Rakshith D, Santosh P, Satish S. 2013 – Isolation and characterization of antimicrobial metabolite producing endophytic *Phomopsis* sp. from *Ficus pumila* Linn. (Moraceae). International Journal of Chemical and Analytical Science 4, 156–160.
Rao DM, Rao UVU, Sudharshanam G. 2006 – Ethno-medico-botanical studies from Rayalaseema region of southern Eastern Ghats, Andhra Pradesh, India. Ethnobotanical Leaflets 1, 21.

Raviraja NS. 2005 – Fungal endophytes in five medicinal plant species from Kudremukh Range, Western Ghats of India. Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms 45, 230–235

Rodriguez RJ, Henson J, Van Volkenburgh E et al. 2008 – Stress tolerance in plants via habitatadapted symbiosis. ISME Journal 2, 404–416

Singh B, Kaur A. 2016 – Antidiabetic potential of a peptide isolated from an endophytic *Aspergillus awamori*. Journal of applied microbiology 120, 301–311.

Sorres J, Nirma C, Touré S, Eparvier V, Stien D. 2015 – Two new isopimarane diterpenoids from the endophytic fungus *Xylaria* sp. SNB-GTC2501. Tetrahedron letters 56, 4596–4598.

Sridhar KR. 2019 – Diversity, ecology and significance of fungal endophytes. In: Jha S, editor. Endophytes and secondary metabolites. Springer Nature Switzerland AG, pp 62–99.

Strobel G, Daisy B. 2003 – Bioprospecting for microbial endophytes and their natural products. - Microbiology and molecular biology reviews 67, 491–502.

Strobel G, Ford E, Worapong J, Harper JK et al. 2002 – Isopestacin, an isobenzofuranone from *Pestalotiopsis microspora*, possessing antifungal and antioxidant activities. Phytochemistry 60, 179–183.

Sunaryanto R, Mahsunah AH. 2014 – Isolation, purification, and characterization of antimicrobial substances from endophytic actinomycetes. Makara Journal of Science 17, 87–92.

Tansuwan S, Pornpakakul S, Roengsumran S, Petsom A et al. 2007 – Antimalarial benzoquinones from an endophytic fungus, *Xylaria* sp. Journal of natural products 70, 1620–1623.

Tibpromma S, Hyde KD, Bhat JD, Mortimer PE et al. 2018 – Identification of endophytic fungi from leaves of Pandanaceae based on their morphotypes and DNA sequence data from southern Thailand. MycoKeys 33, 25.

Venugopalan A, Srivastava S. 2015 – Endophytes as in vitro production platforms of high value plant secondary metabolites. Biotechnology advances 33, 873–887.

Vieira WA, Michereff SJ, de Morais MA, Hyde KD, Câmara MP. 2014 – Endophytic species of *Colletotrichum* associated with mango in northeastern Brazil. Fungal diversity 67, 181–202.