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Endophytic fungi of medicinal plants from the Indo-Gangetic Plains: Diversity, bioactive potential, and what it means for drug discovery

Jyoti Verma
Department of Life Sciences, HRIT University, Ghaziabad, Uttar Pradesh, India

Abstract
Endophytic fungi live inside plant tissues -- roots, stems, leaves -- without causing any visible disease. Honestly, that alone is fascinating. But what makes them medically important is what they produce: potent secondary metabolites, some of which have turned out to be drugs or drug leads. In this study, we surveyed endophytic fungi from 14 medicinal plant species growing in and around Ghaziabad district over two fieldwork phases (2021--2023 and 2024--2025). In all, we isolated and identified 312 strains belonging to 38 genera. Colletotrichum, Aspergillus, and Penicillium showed up most often -- together, they made up nearly half of everything we found (47.1%). ITS-rDNA sequencing sorted 89% of strains to species level. Eleven strains didn't match anything in the databases well enough -- we're treating those as potentially new taxa.
We screened everything against six pathogens, including MRSA and drug-resistant Candida. About 19.6% of strains showed real activity against at least one target. The standout was isolate HRIT-EF-47, pulled from Ocimum sanctum roots. Its MIC against MRSA came in at 3.1 µg/mL -- that's on par with linezolid, the standard drug. HPLC-MS work on 18 priority strains turned up 74 secondary metabolites, nine of them never described before; four of those nine are now fully characterised by NMR. Separately, three Curcuma longa endophytes (all Aspergillus terreus) knocked down TNF-a by 55--68% in macrophage assays -- and there was no curcumin in their profiles, which surprised us.
There's a conservation angle here too. Between Phase I and Phase II, Shannon diversity dropped from H' = 2.63 to 2.19 -- a 16.7% fall. That correlated strongly with NDVI decline at the same sites (r = 0.74). As the plant habitats degrade, the endophyte communities go with them. Given how much pharmacological potential sits in these fungi, that's not just an ecological problem.

Keywords: Endophytic fungi, medicinal plants, secondary metabolites, MRSA, drug discovery, Indo-Gangetic Plains, ITS sequencing, conservation mycology

Introduction

Inside every healthy plant, hidden from the naked eye, there's a microbial world. Endophytic fungi colonise intercellular spaces in leaves, stems, bark, and roots -- and do so without triggering disease in the host. For a long time, they were overlooked. Then in 1993, Stierle and colleagues pulled off something remarkable: they isolated a fungus called Taxomyces andreanae from Pacific yew bark and showed it was independently making paclitaxel, one of the most important cancer drugs at the time [1]. That finding changed everything. Suddenly endophytes weren't just curious biology -- they were pharmaceutical targets [2].

The reason this matters so much right now is straightforward: we're running out of antibiotics. WHO data puts drug-resistant infections at over 1.27 million deaths a year, globally [3]. Meanwhile, the pipeline for genuinely new antibiotic classes has nearly dried up -- fewer than 15 new classes were approved between 2000 and 2023, and most were modifications of old scaffolds rather than structurally new entities [4]. Fungal endophytes are attractive partly because so little of their diversity has been explored. Current estimates say less than 5% has been studied at even a basic level [5].

India's Indo-Gangetic plains are an interesting place to look, though they've been largely ignored in endophyte research. This belt has been growing and using medicinal plants for more than three thousand years -- Ocimum sanctum, Tinospora cordifolia, Azadirachta indica, Curcuma longa -- all documented in Ayurvedic texts going back millennia [6]. But while researchers in the Himalayas and Western Ghats have built up decent endophyte datasets [7, 8], the plains have been mostly skipped. Maybe because they're seen as less botanically exciting. But plants adapted to alkaline soils, high summer heat, and the fluctuating hydrology of the Yamuna basin are likely hosting fungi with equally unusual metabolic tricks.

Our work focused on western Uttar Pradesh -- Ghaziabad, Hapur, Meerut, Bulandshahr. Traditional vaidyas still practice here, and medicinal plants grow in kitchen gardens, agroforestry plots, and along river margins. The aims were: (i) map endophyte diversity across 14 medicinal plant species; (ii) screen for antimicrobial and anti-inflammatory activity against clinically relevant targets; (iii) characterise secondary metabolites from the most active strains; and (iv) check whether ongoing habitat degradation was showing up in the endophyte data, because what we saw on the ground at these sites between visits was not encouraging.

Materials and Methods

1. Study Area and Plant Selection

We worked across two phases: Phase I, July 2021 to March 2023; Phase II, September 2024 to February 2025. The gap was intentional -- it let us compare the same sites across time rather than just piling up isolates from a single visit. Fourteen host species were chosen using two filters: use-value index UV >= 1.2 from our earlier ethnobotanical surveys in the same region, and AYUSH priority species status. Collection sites (n = 14) covered riparian vegetation on the Yamuna and Hindon rivers, mixed agroforestry plots,

kitchen gardens maintained by local vaidyas, and dry deciduous scrub on Ghaziabad's urban edge.

All sites were GPS-tagged. Voucher specimens went to the HRIT University Herbarium (prefix HRIT-H) and were cross-checked against Flora of the Upper Gangetic Plain. Collection followed NBA Access and Benefit Sharing requirements, registration NBA/GZ/2021-25.

2. Isolation of Endophytic Fungi

Healthy-looking leaf, stem, and root segments (2--3 cm) were collected and processed within six hours. Surface sterilisation protocol [9]: 70% ethanol for 90 seconds, 3.5% sodium hypochlorite for four minutes, three washes in sterile distilled water (60 seconds each). The final rinse water was plated on PDA to confirm sterilisation worked -- any sample whose rinse water grew colonies was discarded. Sterilised segments were plated on PDA with chloramphenicol (250 mg/L) to suppress bacteria, incubated at 25 +/- 1-degree C in the dark. Colonies emerging from cut edges over 7--14 days were subcultured until axenic. All isolates stored at -80 degrees C in 20% glycerol, deposited in HRIT's Microbial Culture Repository.

3. Molecular Identification

DNA from five-day mycelium by modified CTAB + bead-beating. ITS1--5.8S--ITS2 amplified with ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR conditions: 94 degrees C 4 min; 35 cycles at 94/55/72 degrees C (30 seconds each); 72 degrees C 7 min extension. Sanger sequencing by Xcelris Genomics, Ahmedabad. Sequences matched against NCBI GenBank and UNITE v9.0. Thresholds: >= 97% = species level; 93--96% = genus level; < 93% = putative novel taxon. Novel sequences submitted to GenBank (accession numbers pending). Phylogenetic trees: MEGA 12, maximum likelihood, GTR+G model, 1,000 bootstrap replicates.

4. Bioactivity Screening

Crude ethyl acetate extracts from 14-day shake cultures (PDB, 120 rpm, 25 degrees C). Broth filtered, partitioned three times against equal volumes of ethyl acetate, dried over anhydrous sodium sulphate, evaporated under reduced pressure. Stored at -20 degrees C until use.

Antimicrobial screening: agar well diffusion against S. aureus MTCC 3160, clinical MRSA (KIMS Hospital, Ghaziabad), E. coli MTCC 739, P. aeruginosa MTCC 2453, C. albicans MTCC 227, and fluconazole-resistant C. albicans (clinical isolate). MICs by broth microdilution in 96-well plates, CLSI M07-A10 [10]. Controls: ciprofloxacin, linezolid, fluconazole; DMSO <= 0.5% as vehicle.

Anti-inflammatory: RAW 264.7 macrophages (ATCC TIB-71), 2 x 10(5) per well, primed with LPS (1 µg/mL, E. coli O111:B4) then co-treated with extract at 12.5, 25, 50, and 100 µg/mL for 24 hours. TNF-a and IL-6 in supernatants by sandwich ELISA (R&D Systems, Minneapolis). MTT assay for viability -- anything below 80% was excluded from interpretation.

5. Metabolite Profiling

Eighteen strains with MIC <= 16 µg/mL or TNF-a inhibition >= 40% were profiled by HPLC-MS. Extracts in methanol at 1 mg/mL, filtered (0.22 µm PVDF), run on Agilent 1290 Infinity II UHPLC + Agilent 6550 Q-TOF (positive and negative ion modes, m/z 100--1700). Column: Zorbax Eclipse Plus C18 (2.1 x 100 mm, 1.8 µm), water-acetonitrile gradient, 0.1% formic acid. Data processed in MZmine 3.4, matched against Natural Products Atlas and ChemSpider. Cosine similarity < 0.80 = putatively novel. Four such compounds taken to 1D/2D NMR at CSIR-CDRI Lucknow on a Bruker Avance NEO 600 MHz.

6. Diversity Analysis and Statistics

Shannon H', species richness, and Sorensen coefficients in R v4.4.1 (vegan package). ANOVA for richness differences across tissue types and host species; post-hoc Tukey HSD at $p < 0.05$. Per-site NDVI from Landsat 8/9 via Google Earth Engine (annual composites, 2015--2024). Pearson's r linked per-site NDVI decline to H' change between phases.

Results

1. Diversity and Composition

Total yield across both phases: 312 strains from 14 hosts. Colonisation rate was 74.6% of tissue segments processed. Molecular ID sorted 278 (89.1%) to species level, 23 (7.4%) to genus only, and flagged 11 (3.5%) as putative new taxa. Collection spans 38 genera and six classes -- Sordariomycetes dominated at 38.5%, followed by Dothideomycetes (27.2%), Eurotiomycetes (18.3%), Leotiomycetes (8.0%), Agaricomycetes (4.5%), and Pezizomycetes (3.5%).

Most frequently isolated genera: Colletotrichum (n = 56, 17.9%), Aspergillus (n = 48, 15.4%), Penicillium (n = 43, 13.8%) -- 47.1% of the whole collection. Azadirachta indica and Ocimum sanctum gave the richest diversity: 52 strains across 14 genera, and 46 strains across 12 genera, respectively. Root tissue outperformed stem and leaf across all hosts ($F_{2,311} = 18.4$, $p < 0.001$), which lines up with what Rodriguez *et al.* reported about the chemical complexity of root-zone environments [11].

Comparing phases: Mean isolates per host dropped from 25.0 (Phase I) to 19.8 (Phase II), $p = 0.032$. Shannon H' fell from 2.63 to 2.19 -- that's 16.7% down. Per-site H' decline correlated with per-site NDVI reduction ($r = 0.74$, $p < 0.01$). As habitat quality fell, the endophyte communities followed.

Table 1: Host plants surveyed and endophyte isolate data

Host Plant	Family	Primary Medicinal Use	Isolates (n)	Genera (n)
Ocimum sanctum L.	Lamiaceae	Antimicrobial, adaptogen	46	12
Azadirachta indica A. Juss.	Meliaceae	Antibacterial, antifungal	52	14
Curcuma longa L.	Zingiberaceae	Anti-inflammatory	38	10
Tinospora cordifolia (Willd.) Hook.f.	Menispermaceae	Immunomodulatory	27	8
Withania somnifera (L.) Dunal	Solanaceae	Adaptogen, anticancer	24	7
Phyllanthus emblica L.	Phyllanthaceae	Antioxidant, hepatoprotective	22	7
Andrographis paniculata (Burm.f.) Nees	Acanthaceae	Antipyretic, anti-inflammatory	21	6

Aloe vera (L.) Burm.f.	Asphodelaceae	Wound healing, antibacterial	19	6
Moringa oleifera Lam.	Moringaceae	Nutritive, antidiabetic	17	5
Calotropis procera (Aiton) W.T. Aiton	Apocynaceae	Analgesic, antimalarial	16	5
Eclipta prostrata (L.) L.	Asteraceae	Hepatoprotective	12	4
Aegle marmelos (L.) Corra	Rutaceae	Antidysenteric, cardioprotective	11	4
Plumbago zeylanica L.	Plumbaginaceae	Anti-inflammatory, antibacterial	4	3
Vernonia cinerea (L.) Less.	Asteraceae	Antipyretic, anthelmintic	3	3

2. Antimicrobial and Antifungal Activity

Of 312 screened strains, 61 (19.6%) produced inhibition zones >= 10 mm against at least one pathogen. Anti-MRSA hits: 29 isolates (9.3%), zones 11--27 mm. The top performer was HRIT-EF-47, from Ocimum sanctum roots -- 27 mm against MRSA, bigger than the linezolid disc control at 22 mm. MIC: 3.1 µg/mL vs. MRSA and 4.8 µg/mL vs. fluconazole-resistant C. albicans. ITS placed HRIT-EF-47 in the Colletotrichum gloeosporioides complex at 94.6% identity -- close enough for genus, but divergent enough that it's probably a distinct lineage.

Gram-negative bacteria were a different story. Only 14 isolates hit >= 10 mm against E. coli or P. aeruginosa, with MICs in the 64--256 µg/mL range -- too high to be practically useful. This fits what others have reported: endophyte-derived compounds tend to be much more active against Gram-positive targets than Gram-negative ones [12].

3. Anti-Inflammatory Results

Three isolates from Curcuma longa roots -- HRIT-EF-88, -89, and -91 -- stood out in the macrophage assay. At 50 µg/mL crude extract, TNF-a suppression was 55.2%, 68.4%, and 61.0% respectively (all $p < 0.001$ vs. LPS control). IL-6 came down 48--61%. Cell viability by MTT stayed above 88% across all doses -- no cytotoxicity issue. All three key out as Aspergillus terreus (99.8% pairwise ITS identity), a species already on record for polyketide anti-inflammatory metabolites including terrein and butyrolactone I [13].

Here's what surprised us: no curcumin, and nothing structurally related, showed up in the HPLC profiles of any of the three strains. So, the activity we measured came entirely from fungal chemistry, not plant residues in the tissue. Whether that reflects co-evolutionary convergence between host and endophyte chemistry -- what some call metabolic mirroring [18] -- is an open question, but it's one worth chasing.

4. Secondary Metabolite Characterisation

HPLC-MS of 18 priority strains resolved 74 metabolite features. Sixty-five matched database entries at cosine >= 0.80 -- known scaffolds like terrein, cytochalasins B and D, alternariol, phomactin A, and various xanthones. Nine features from five strains matched nothing in either database. Four of those nine went to NMR.

The most structurally novel was HRIT-M-1 from HRIT-EF-47. High-resolution MS gave a molecular formula of C18H22O5 (MW 322.36 g/mol; [M+H] + at m/z 323.14, 1.2 ppm error). 2D NMR worked out a bicyclic polyketide in which an alpha-pyrone ring is fused to a dihydropyran -- a connectivity we haven't found described in fungal natural products before. QSAR modelling via SwissTargetPrediction predicted moderate affinity for PBP2a, the main beta-lactam resistance protein in MRSA [14]. That's computational, not bench-confirmed, but it gives us a testable hypothesis.

Table 2: Bioactivity summary for selected endophyte isolates

Isolate ID	Host Species	MIC vs. MRSA (µg/mL)	TNF-a Inhibition (%)	Novel Compound
HRIT-EF-47	*Ocimum sanctum*	3.1	--	HRIT-M-1 (bicyclic polyketide)
HRIT-EF-88	*Curcuma longa*	64	68.4	--
HRIT-EF-89	*Curcuma longa*	128	55.2	--
HRIT-EF-91	*Curcuma longa*	32	61.0	Butyrolactone I analogue
HRIT-EF-12	*Azadirachta indica*	8.0	42.5	HRIT-M-2 (xanthone deriv.)
HRIT-EF-31	*Withania somnifera*	16.0	38.0	HRIT-M-3 (indole alkaloid)
HRIT-EF-55	*Tinospora cordifolia*	12.5	44.7	--
HRIT-EF-70	*Phyllanthus emblica*	4.8 (C. alb.)	--	HRIT-M-4 (sesquiterpenoid)

Discussion

Three hundred and twelve characterised endophytic strains from Indo-Gangetic medicinal plants -- as far as we can tell, that's the largest such collection from this specific region published so far. Colletotrichum and Aspergillus dominating the assemblage makes sense for a subtropical agroforestry landscape [15], but 3.5% putative novel taxa is higher than we expected, especially using a conservative ITS threshold. One in roughly 30 isolates didn't match anything catalogued. The plains aren't as mycologically well-documented as their prominence in agricultural literature might suggest.

The MRSA data are the most practically significant part of this work. ICMR surveillance puts MRSA at roughly 47% of S. aureus hospital isolates nationally in 2022, with bloodstream infection fatality at 28--34% in tertiary care [16]. We don't have many good new drugs for this. HRIT-EF-47's MIC of 3.1 µg/mL against a clinical MRSA isolate is a strong opening number -- Nair and Padmavathy, surveying 200 Himalayan conifer endophytes, found MICs below 25 µg/mL against S. aureus to be uncommon [17]. Getting from an active extract to something clinical is a long haul, but HRIT-M-1's structural novelty and predicted PBP2a affinity [14] make the next steps -- fermentation scale-up, compound purification, mouse infection model -- worth funding.

The C. longa endophyte results needed a double-check. Everyone working with this plant knows curcumin -- it's the headline phytochemical. When three endophytes from C. longa roots showed 55--68% TNF-a suppression, the obvious worry was contamination with plant-derived curcuminoids. HPLC said no. The profiles of HRIT-EF-88, -89, and -91 had nothing in the curcuminoid region.

Whatever's responsible for the anti-inflammatory activity is purely fungal in origin. This kind of apparent metabolic convergence between host and endophyte has been reported elsewhere -- Kusari *et al*. documented it with camptothecin in Camptotheca endophytes [18] -- but it's not routine, and the structural basis here is worth working out properly.

The biodiversity decline between survey phases is the finding that probably has the broadest implications. A 16.7% drop in Shannon H' within three to four years, correlating tightly with NDVI loss at the same sites (r = 0.74), is a clear signal, not noise. Vandenkoornhuyse *et al*. make the case that plant microbiomes are integral parts of the plant holobiont, not incidental passengers [19]. If that framing is right, losing endophyte diversity is losing functional biology, not just pharmacological opportunity. And because culture-based methods probably capture 1--10% of the real community [20], the actual loss is likely larger than our H' numbers show.

Two limitations to note honestly: our MIC values are for crude extracts, not pure compounds, so direct comparison with published pure-compound values is imprecise. And the macrophage results are *in vitro* only. Both gaps need to be filled before any of this moves toward application.

Conclusion

Endophytic fungi from medicinal plants of the Indo-Gangetic plains are a pharmacological resource that's barely been touched. This study characterised 312 strains, turned up nine previously undescribed metabolites, and identified one isolate -- HRIT-EF-47 from Ocimum sanctum -- producing something novel with serious anti-MRSA activity *in vitro*. At the same time, the data make clear this resource is under active pressure. Endophyte richness is falling in step with habitat degradation, and the two problems have to be addressed together. Protecting the riparian corridors and agroforestry zones of western Uttar Pradesh isn't only an ecological argument -- it's keeping a chemical library intact. HRIT University's ethnopharmacology programme is positioned to drive this forward, and that's what we plan to do.

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