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ISOLATION, MOLECULAR IDENTIFICATION AND ANTIMICROBIAL ACTIVITY OF ASPERGILLUS VERSICOLOR AND OTHER ENDOPHYTIC FUNGI FROM THUNBERGIA ERECTA

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Abstract

Endophytic fungi are non-pathogenic microorganisms residing within healthy plant tissues and are known to produce diverse bioactive secondary metabolites. This study focused on isolating and characterizing endophytic fungi from Thunbergia erecta, a medicinal and ornamental plant. Six morphologically distinct fungal isolates were selected for analysis. Molecular identification confirmed Aspergillus versicolor (TE2) and Aspergillus micronesiensis (TE4), while Penicillium sp.1 (TE3) and Penicillium sp.2 (TE9) were identified based on morphology. TE7 and TE8 remained unidentified due to lack of molecular data. Antimicrobial activity was evaluated against four bacterial pathogens (Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus) and four fungal pathogens (Candida albicans, Aspergillus fumigatus, Fusarium oxysporum, and Penicillium chrysogenum). A. versicolor (TE2) exhibited the strongest antibacterial activity, with inhibition zones of 24 mm against B. subtilis and 22 mm against other bacteria, exceeding Gentamicin (18 mm). TE7 showed comparable antibacterial activity, while Penicillium sp.1, Aspergillus micronesiensis and Penicillium sp.2 demonstrated moderate activity. TE8 exhibited relatively lower antibacterial effects. In antifungal assays, A. versicolor (TE2) showed significant inhibition against A. fumigatus (12 mm), C. albicans and F. oxysporum (10 mm), and P. chrysogenum (8 mm). TE7 and Penicillium sp.2 displayed moderate antifungal activity, while TE8 had limited effects. Penicillium sp.1 showed no antifungal inhibition. These findings highlight A. versicolor (TE2) as a promising source of antimicrobial compounds and encourage further research on T. erecta endophytes.

Keywords: Thunbergia erecta, endophytic fungi, antimicrobial activity, Aspergillus versicolor, ITS rDNA sequencing..

1. Introduction

Medicinal plants have been used for centuries for their therapeutic properties, providing remedies for many human ailments (Schmeda-Hirschmann *et al*., 2005). Recent studies have emphasized the important role that plant-associated microorganisms, especially endophytes, play in enhancing these medicinal benefits (Hadadi *et al*., 2020). The term *endophyte* was first introduced by the German scientist Heinrich Anton de Bary in 1884 to refer to bacteria or fungi that live inside plant tissues without causing obvious harm to the host (Shady *et al*., 2023). These

organisms form a symbiotic relationship with their host plants: the plant supplies shelter and nutrients, while the endophytes produce a range of secondary metabolites that help the plant cope with various biotic and abiotic stresses (Tan *et al*., 2012).

Endophytic fungi are known to synthesize many bioactive compounds, including alkaloids, terpenoids, steroids, quinones, isocoumarins, flavonoids, phenols, phenolic acids, and peptides (Wang *et al*., 2014). These secondary metabolites have industrial, antibacterial, and anticancer potential. For example, *Taxomyces andreanae* produces Taxol, a well-known anticancer drug, while *Cryptosporiopsis quercina* synthesizes Cryptocandin, a novel antifungal agent. It has been estimated that more than 80% of endophytic fungi generates bioactive secondary metabolites with diverse biological effects, including fungicidal, antibacterial, and herbicidal activities (Stierle *et al.*,1993). Because of this richness in novel compounds, endophytes are promising sources for developing new pharmaceuticals and industrial agents (Kumar *et al*., 2023).

The rise of multidrug-resistant pathogens has increased the urgency to discover new antimicrobial agents. Misuse of antibiotics, inadequate hygiene, growing numbers of immunocompromised individuals, and delays in diagnosing infections all contribute to antibiotic resistance worldwide (Strobel, 2003). In this crisis, natural compounds especially those from plants, fungi, and bacteria stand out as promising alternatives, as many have unique modes of action and may be less likely to provoke resistance (Ahmed *et al*., 2023). Endophytes, being reservoirs of diverse bioactive compounds, are receiving considerable attention in this search. Many secondary metabolites from endophytic fungi, such as alkaloids, terpenoids, coumarins, and lignans, have demonstrated antimicrobial activity (Schulz *et al.,* 2002). In some cases, these fungal-derived compounds match or exceed those produced by their host plants. Endophytes also contribute to plant growth: they synthesize phytohormones and enhance tolerance to stresses like salinity or heavy metal exposure. This mutualistic relationship confers both ecological and pharmaceutical benefits (Bhat *et al*., 2021).

Thunbergia erecta, commonly known as bush clock vine or king's-mantle, is a flowering plant in the family Acanthaceae, widely cultivated for its ornamental value due to its attractive blue to violet flowers. Beyond its horticultural appeal, *T. erecta* has been reported to possess notable medicinal properties. Phytochemical and pharmacological studies have shown that leaf extracts exhibit sedative, anxiolytic, analgesic, and anti-inflammatory activities in animal models (Chatterjee *et al*., 2015; Refaey *et al*., 2021). Moreover, some preliminary reports suggest that *T. erecta* extracts may also have antimicrobial potential, although the specific compounds and mechanisms remain underexplored. These bioactivities suggest the presence of diverse secondary metabolites, making *T. erecta* a promising candidate for bioprospecting studies particularly the investigation of its endophytic fungi for novel antimicrobial agents.

The present study aims to isolate endophytic fungi from different parts of the *Thunbergia erecta* plant collected in Mysore, Karnataka, India. Secondary metabolites will be extracted from the isolated fungal strains and evaluate for their antimicrobial properties. The goal is identify novel bioactive compounds with potential applications in industry and medicine. By exploring both molecular and bioactive characterisitcs of these endophytes, this research hopes to contribute to the development of new antimicrobial agents.

2. Materials and Methods

2.1 Collection of plant materials

Healthy and mature samples of *Thunbergia erecta* (Acanthaceae) were collected from Mysore, Karnataka, in October 2022. The samples were cut, placed in sterile polythene bags, and transported to the laboratory, where they were thoroughly washed prior to analysis. Identification was carried out based on morphological characteristics and confirmed by the Botanical Survey of India, Coimbatore.

2.2 Surface sterilization and isolation of fungal endophytes

Leaves, stems, and flowers were washed under running tap water for 10 minutes and air-dried. The materials were then cut into smaller sections and surface-sterilized by sequential immersion in 70% ethanol for 1 minute, followed by 1.2% sodium hypochlorite for 2 minutes, and finally rinsed three times with sterile distilled water. Sterility was confirmed by plating the final rinse onto Water Agar (WA) medium, as described by Giang *et al., (*2021).

Following surface sterilization, the plant materials were dried on sterile blotting paper under laminar airflow. Using a sterile blade, small segments (1.0 × 1.0 cm) were excised from the leaves, stems, and flowers. These segments were aseptically placed at equal distances onto Petri dishes containing Water Agar (WA) medium. The plates were sealed with Parafilm™ and incubated at 27 ± 2°C under a 12-hour light/12-hour dark photoperiod for 4 to 6 weeks. Fungal growth was monitored periodically, and emerging hyphal tips were carefully transferred to fresh Potato Dextrose Agar (PDA) slants using a sterile fine-tipped needle. The subcultured isolates were incubated at 27 ± 2°C for 10–15 days and subsequently stored at 4°C for future use.

2.3 Identification of endophytic fungi

Morphological identification

Endophytic fungi were cultured on Potato Dextrose Agar (PDA) and incubated for seven days. Colony morphology (growth pattern, texture, color) and spore characteristics were examined using the tease mount method with lactophenol cotton blue under 40× magnification. Identification was based on mycelial and reproductive structures using standard manuals (Barnett and Hunter, 1998; Watanabe, 2002).

Molecular identification and phylogenetic analysis

For molecular identification of fungal species, genomic DNA was extracted from the cultured isolates. Actively growing, morphologically distinct hyphal tips were transferred to Potato Dextrose Broth (PDB) and incubated at 27 ± 2°C for 7–10 days. The resulting fungal biomass was collected, freeze-dried, and finely ground in liquid nitrogen. The powdered mycelial tissue was then stored at –20°C until further use for DNA extraction.

DNA extraction

Genomic DNA was extracted from freeze-dried fungal biomass using the CTAB method with minor modifications (Ausubel *et al.,* 1994). Fungal tissue was ground with extraction buffer and incubated at 55 °C for 1 hour, followed by heat treatment at 95 °C for 20 minutes. After centrifugation, the supernatant was treated with chloroform:isoamyl alcohol (24:1), and RNase was added. DNA was precipitated using cold isopropanol, washed with 70% ethanol, air-dried, and dissolved in 1X Tris-EDTA buffer for further analysis.

DNA amplification and sequencing

The ITS region of fungal DNA was amplified using ITS1 and ITS4 primers with a PCR 18 kit (Barcode Biosciences, Bengaluru). PCR conditions included initial denaturation at 94 °C

for 2 min, followed by 35 cycles of denaturation (94 °C, 1 min), annealing (47 °C, 15 sec), extension (72 °C, 30 sec), and a final extension at 72 °C for 10 min. PCR products were visualized on 1.0% agarose gel stained with ethidium bromide and documented using a GelDoc XRT system (Bio-Rad, USA). Purification and sequencing were conducted by Barcode Biosciences. The obtained sequences were analyzed using the BLAST tool against the NCBI GenBank database .

Phylogenetic analysis

Sequences of highly bioactive isolates were aligned with the top ten BLAST hits using ClustalW . A phylogenetic tree was constructed using MEGA versions 11 based on a distance matrix.

2.4 Extraction of secondary metabolites

Fungal isolates were cultured in 150 mL of potato dextrose broth (PDB) in 250 mL autoclaved Erlenmeyer flasks and incubated at 29 ± 1 °C, 180 rpm, for 21 days, following a modified method of Nisa *et al.*, (2020). After incubation, the biomass was separated using Whatman filter paper, and the culture filtrate was extracted with ethyl acetate in a separating funnel. The organic layer was concentrated using a rotary evaporator to obtain the crude extract, which was stored at 4–5 °C. For biological assays, part of the extract was dissolved in DMSO at 4 mg/mL, and the remainder was reserved for chemical analysis.

2.5 Antimicrobial activity of secondary metabolites

The antimicrobial activity of the fungal extract was evaluated using the agar well diffusion method with slight modifications (Brantner *et al.,* 1994; Magaldi *et al.,* 2004).

For antibacterial testing, nutrient agar was prepared and sterilized by autoclaving at 121 °C for 15 minutes. After cooling to 45–50 °C, 25 mL of the sterile medium was poured into sterile Petri dishes and allowed to solidify under aseptic conditions. Fresh cultures of selected bacterial strains Gram-positive *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (168), and Gram-negative *Escherichia coli* (MTCC 118) and *Pseudomonas aeruginosa* (MTCC 424) were uniformly spread on the surface of the agar using sterile cotton swabs. Wells of 4 mm diameter were aseptically punched into the agar using a sterile cork borer. Each well was filled with 100 µL of fungal extract (50 mg/mL in DMSO). Gentamicin (1 mg/mL in DMSO) served as positive control, while pure DMSO was used as negative control. The plates were incubated at 37 °C for 24 hours, after which zones of inhibition (ZOI) were measured in millimeters using a vernier caliper.

For antifungal testing, fresh fungal cultures *Candida albicans* ATCC 90028, *Aspergillus fumigatus* MTCC 9657, *Fusarium oxysporum* MTCC 1755, and *Penicillium chrysogenum* MTCC 108 were uniformly spread on potato dextrose agar (PDA) plates. Wells were aseptically punched and filled with 100 µL of fungal extract (10 mg/mL in DMSO). Fluconazole (1 mg/mL) was used as positive control. The plates were incubated at 30 °C for 2–3 days, and inhibition zones were measured in millimeters.

2.6 Statistical analysis

Mean, standard deviation and descriptive statistics were performed using PRISM software (PRISM 8 Version 8.0.2). Comparison of mean and significance was determined by one-way ANOVA with Geisser-Greenhouse model. Multiple comparison was made using Tukey's test and the significance level was measured with $p<0.05$.

3. Results

3.1 Isolation of endophytic fungi from *Thunbegia erecta*

A total of several endophytic fungal isolates were obtained from surface-sterilized leaves, stems, and flowers of *Thunbergia erecta* after incubation on Water Agar (WA) medium. Fungal growth began to emerge from internal tissues within 7 to 14 days of incubation. All isolates were successfully subcultured onto Potato Dextrose Agar (PDA), where they exhibited diverse colony morphologies. Among the isolated fungi, six morphologically distinct and fast-growing isolates were selected for further characterization. These were designated as TE2, TE3, TE4, TE7, TE8 and TE9. TE2 andTE9 was isolated from leaf tissue, TE3 , TE7, and TE8 from stem tissue, and TE4 from flower tissue. These isolates showed dominant growth patterns and unique colony features compared to others on the isolation plates (Figure 1; Table 1). The selected isolates were preserved on PDA slants at 4 °C and subjected to molecular identification using ITS rDNA sequencing.

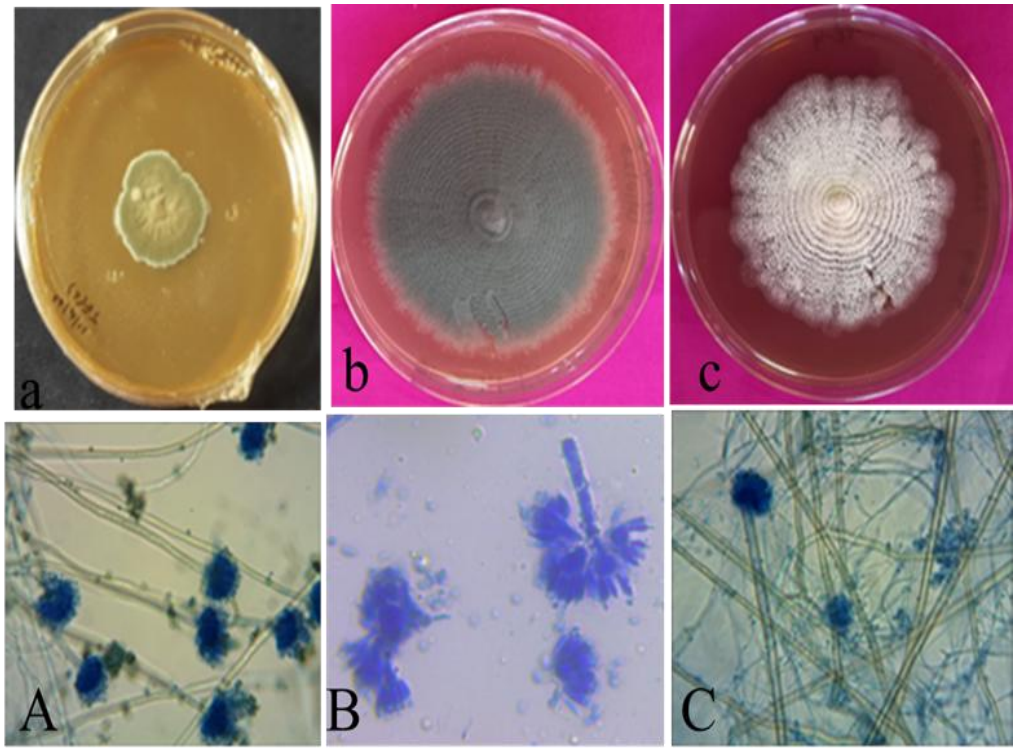

Figure: 1 Morphological and microscopic observation of fungal endophytes isolated from *T.erecta* on PDA plates (a-A) *Aspergillus versicolor*, (b-B) *Penicillium sp* and (c-C) *Aspergillus micronesienis*

Table 1. Colony morphology and microscopic presentation of isolated endophytic fungi

Isolated Code	Colony Morphology (PDA)	Microscopic Features	Tentative Identification
TE2	Dense, velvety, greenish-blue surface; pale yellow reverse	Septate hyphae; uniseriate conidiophores; globose, finely roughened conidia	*Aspergillus versicolor*
TE3	Fast-growing, Bluish-green colonies with white margins; cream-colored reverse	Brush-like conidiophores; chains of spherical conidia arranged basipetally	*Penicillium* sp.1
TE4	Flat, dense, white to cream-colored colonies; powdery texture	Biseriate conidial heads; phialides covering entire vesicle; small, globose, slightly roughened conidia	*Aspergillus micronesiensis*
TE7	Methypowdery colonies	-	Unidentified
TE8	Greyish cement colonies	-	Unidentified
TE9	Fast-growing, Bluish-green colonies with white margins; cream-colored reverse	Brush-like conidiophores; chains of spherical conidia arranged basipetally	*Penicillium* sp.2

3.2 Molecular identification of endophytic fungi

Molecular identification of two endophytic fungal isolates, TE2 and TE4, was conducted by amplifying and sequencing the internal transcribed spacer (ITS) region of ribosomal DNA using universal primers ITS1 and ITS4. The sequences were analyzed using the BLAST tool against the NCBI GenBank database for species-level identification.

Isolate TE2 showed 100% similarity with *Aspergillus versicolor*, and the sequence was submitted to GenBank under the accession number PQ057012. Similarly, TE4 also showed 100% similarity with *Aspergillus micronesiensis*, with the sequence submitted under GenBank accession number PQ056130. These results strongly support morphological identification.

Due to resource constraints, molecular identification for isolate TE3 was not performed. However, based on morphological characteristics, TE3 and TE9 was tentatively identified as belonging to the genus *Penicillium*. TE7 and TE8 Unidentified.

A phylogenetic tree was constructed using the neighbor-joining method in MEGA 11 software to further validate the molecular identification. Bootstrap analysis with 1000 replicates confirmed the close clustering of TE2 with their respective reference strains from GenBank (Figure 2).

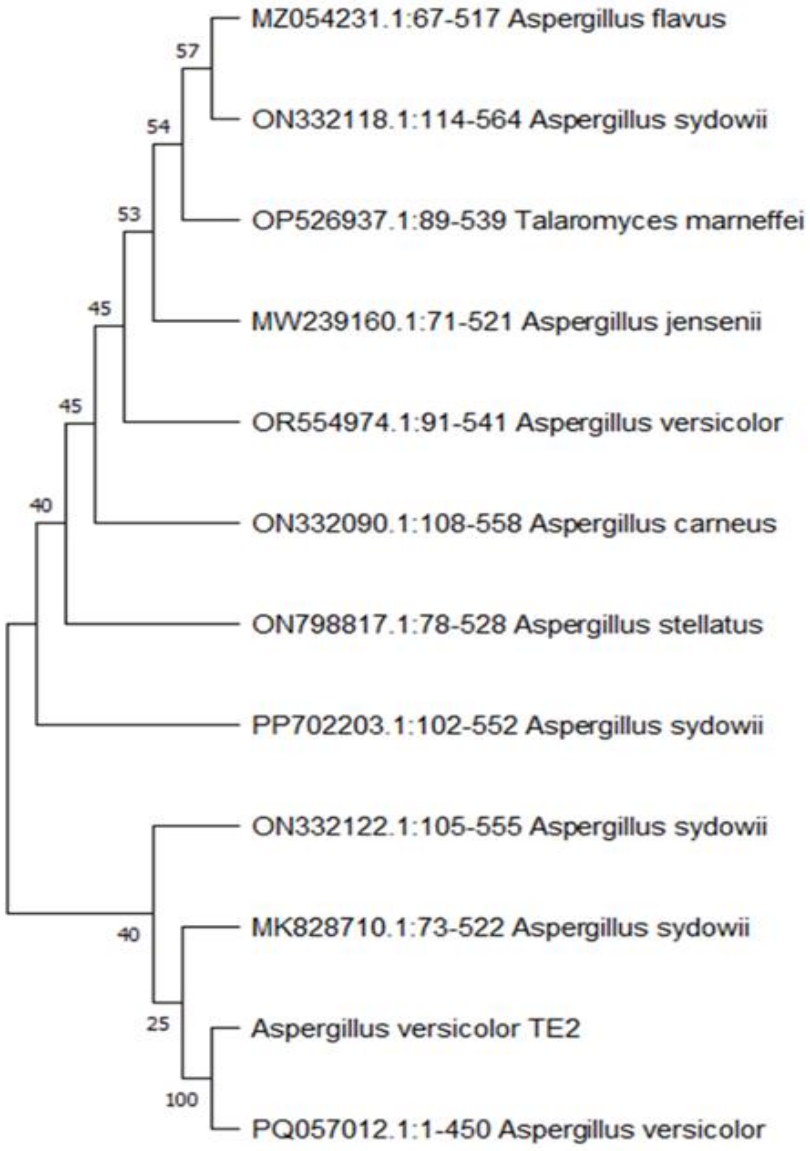

Figure 2 Optimum phylogenetic tree constructed in MEGA -11, by neighbor-joining method and evolutionary distance computed using the maximum composite likelihood method (NJ-MCL) obtained from ITS of TE2 which confirmed to *be Aspergillus versicolor.*

3.3 Antibacterial activity of crude extracts

In the *T. erecta* group, *Aspergillus versicolor* (TE2) stood out by exhibiting the highest antibacterial activity across all tested organisms. It recorded inhibition zones of 22 ± 0.00 mm for *E. coli*, 22 ± 0.09 mm for *P. aeruginosa*, 24 ± 0.00 mm for *B. subtilis*, and 22 ± 0.09 mm for *S. aureus*. The unidentified fungus TE7 also showed strong antibacterial effects, particularly against *E. coli* and *S. aureus* (22 ± 0.45 mm for both), and comparable inhibition against *B. subtilis* and *P. aeruginosa*. *Penicillium* sp1 (TE3) and *Penicillium* sp2 (TE9) demonstrated moderate antibacterial activity, with inhibition zones slightly higher than or similar to the standard antibiotic. In contrast, *Aspergillus micronesienis* TE4 exhibited relatively lower antibacterial activity, with inhibition zones of 20 ± 0.54 mm for *E. coli*, 18 ± 0.63 mm for *P. aeruginosa*, 18 ± 0.36 mm for *B. subtilis*, and 18 ± 0.63 mm for *S. aureus*. While less potent than other *T. erecta*

extract, TE4 still showed activity comparable to the antibiotic control. Unidentified fungus TE8 also displayed consistent antibacterial activity, particularly against *S. aureus* (22 ± 0.36 mm). It showed uniform inhibition zones of 20 mm against *E. coli*, *P. aeruginosa*, and *B. subtilis*, indicating a balanced but moderate spectrum of antibacterial effects.

These results suggest that the endophytic fungi isolated from *T. erecta*, particularly *A. versicolor*, possess promising antibacterial properties and may serve as potential sources of bioactive compounds.

Table 2: Antibacterial activity (Zone of Inhibition in mm) of ethyl acetate extracts from endophytic fungi isolated from *Thunbergia erecta*

SL.NO	Name of the fungi	Isolate no	Zone of inhibition (mm in diameter)			
			E. coli	*P.aeruginosa*	*B.subtilis*	*S.aureus*
1	*Aspergillus versicolor*	TE2	22 ± 0.00^{c}	22 ± 0.09^{c}	24 ± 0.00^{d}	22 ± 0.09^{d}
2	*Penicillium* sp1	TE3	20 ± 0.18^{b}	20 ± 0.18^{b}	20 ± 0.54^{b}	20 ± 0.09^{c}
3	*Aspergillus micronesienis*	TE4	20 ± 0.54^{b}	18 ± 0.63^{a}	18 ± 0.36^{a}	18 ± 0.63^{b}
4	Unidentified	TE7	22 ± 0.45^{c}	20 ± 0.27^{b}	22 ± 0.45^{c}	22 ± 0.45^{d}
5	Unidentified	TE8	18 ± 0.36^{a}	20 ± 0.54^{b}	18 ± 0.18^{a}	15 ± 0.27^{a}
6	*Penicillium* sp2	TE9	20 ± 0.09^{b}	20 ± 0.00^{b}	20 ± 0.09^{b}	20 ± 0.54^{c}
7	Gentamicin	Antibiotic	16 ± 0.09^{c}	18 ± 0.09^{a}	18 ± 0.01^{a}	18 ± 0.01^{b}

Values are mean ± SD (n=2); Different alphabet in the same column are significantly different ($p<0.05$).

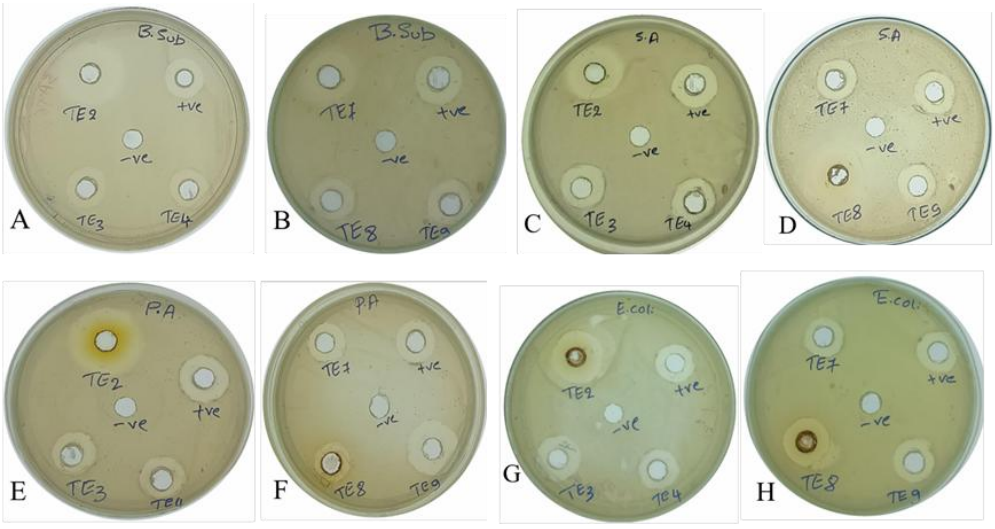

Figure 3. Antibacterial activity of *Aspergillus versicolor* (TE2), *Penicillium* sp1 (TE3), *Aspergillus micronesienis* (TE4), Unidentified (TE7), Unidentified (TE8) *and Penicillium* sp2 TE9 *ethyl acetate extract against* (A-B) B. *subtilis* 168, (C-D) *S. aureus* MTCC 96, (E-F) *P. aeruginosa* MTCC 424 *and* (G-H) *E. coli* MTCC 118,+ ve control gentamycin and - ve control (DMSO).

3.4 Antifungal activity of crude extracts

The ethyl acetate extracts of the six endophytic fungi *Aspergillus versicolor*(TE2), *Penicillium* sp1 (TE3) and *Aspergillus micronesienis* (TE4), TE7,TE8 and *Penicillium* sp2(TE9) were tested for antifungal activity against **four fungal pathogens** *Candida albicans*, *Aspergillus*

fumigatus, *Fusarium oxysporum*, and *Penicillium chrysogenum*. The zone of inhibition values are presented in Table 3 and Figure 4.

In the *T.erecta* , *Aspergillus versicolor* TE2 showed broad-spectrum activity against all four pathogens, with zones ranging from 8 ± 0.18 mm (*P. chrysogenum*) to 12 ± 0.54 mm (*A. fumigatus*). *Penicillium* sp1 (TE3)was effective against *C. albicans* and *P. chrysogenum* but inactive against the remaining two fungi. *Aspergillus micronesienis* (TE4)and Unidentified fungus(TE7)showed inhibition against *C. albicans* and *A. fumigatus*, with additional activity against *F. oxysporum* or *P. chrysogenum*, respectively. Unidentified fungus (TE8) showed limited activity, only inhibiting *C. albicans* and *P. chrysogenum*. *Penicillium* sp2(TE9)displayed weak inhibition against *C. albicans* and *F. oxysporum*, with no activity against the remaining fungi. The standard antibiotic again demonstrated superior antifungal activity, with inhibition zones of 24 ± 0.09 mm (*C. albicans*), 18 ± 0.18 mm (*A. fumigatus*), 18 ± 0.54 mm (*F. oxysporum*), and 20 ± 0.09 mm (*P. chrysogenum*).

When compared to the standard antifungal drug, **Fluconazole**, which showed inhibition zones ranging from 18 ± 0.18 mm to 24 ± 0.09 mm, the activity of the fungal extracts was relatively lower. However, the results indicate that the endophytic fungi from *T. erecta* possess moderate antifungal properties and may serve as potential sources of bioactive antifungal compounds.

Table 3: Antifungal activity (Zone of Inhibition in mm) of ethyl acetate extracts from endophytic fungi isolated from *Thunbergia erecta*

Sl.no	Name of the Fungi	Isolate No	Zone of inhibition (mm in diameter)			
			C. albicans	*A. fumigatus*	*F. oxysporium*	*P. chrysogenum*
1	*Aspergillus versicolor*	TE2	10 ±0.18	12 ±0.54	10 ±0.18	8 ±0.18
2	*Penicillium* sp1	TE3	10 ±0.45	-	-	8 ±0.09
3	*Aspergillus micronesienis*	TE4	10 ±0.36	12 ±0.72	8 ±0.09	-
4	Unidentified	TE7	10 ±0.18	12 ±0.63	-	08 ±0.54
5	Unidentified	TE8	10 ±0.09	-	08 ±0.18	- -
6	*Penicillium* sp2	TE9	10 ±0.36	-	-	08 ±0.18
4	Fluconazole	Antibiotic	24 ±0.09	18 ±0.18	18 ±0.54	20 ±0.09

Values are mean ± SD (n=2); There was no statistical difference ($p>0.05$) between the sample. However, significant difference ($p<0.05$) was observed with respect to standard antibiotic.

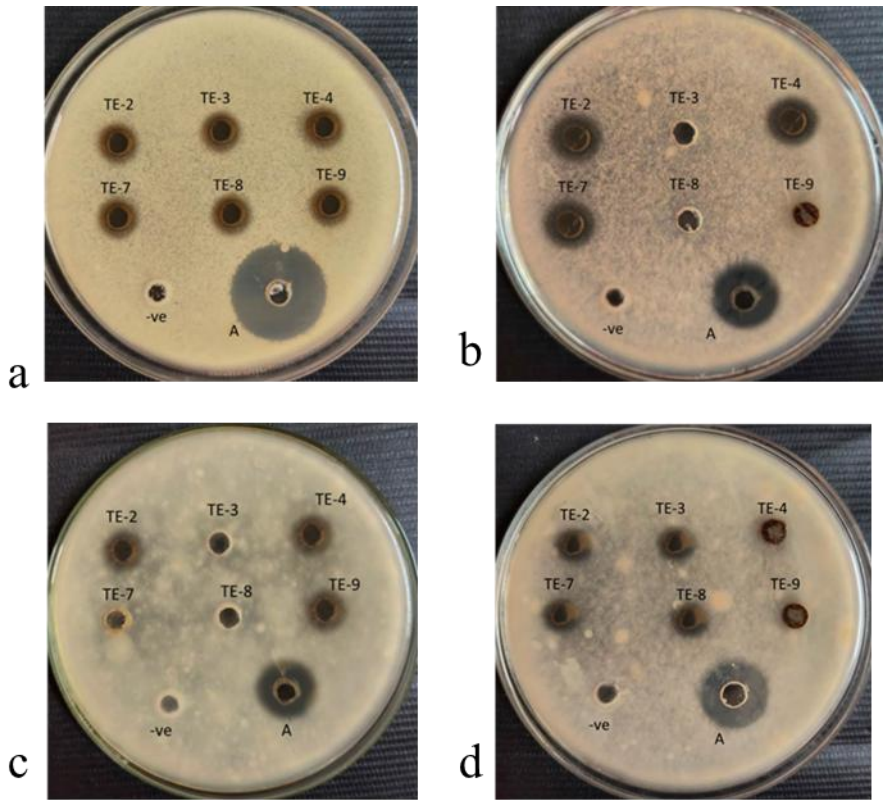

Figure 4.Antifungal activity of *Aspergillus versicolor* (TE2), *Penicillium* sp1(TE3), *Aspergillus micronesienis* (TE4), Unidentified (TE7), Unidentified (TE8) *and Penicillium* sp2(TE9) *ethyl acetate extract against* a) *C. albicans*, b) *A. fumigatus*, c) *F. oxysporium,* d) P. *chrysogenum*

A: Antibiotic Fluconazole and -ve Negative control (DMSO).

4. Discussion

The successful isolation of endophytic fungi from surface-sterilized tissues of *Thunbergia erecta* highlights its potential as a host of diverse microbial species. Among the six dominant isolates (TE2, TE3, TE4, TE7, TE8, and TE9), TE2 and TE4 were identified via ITS sequencing as *Aspergillus versicolor* and *Aspergillus micronesiensis*, respectively, while TE3 and TE9 were tentatively classified as *Penicillium* species. TE7 and TE8 remain unidentified but exhibited notable bioactivity. The search for new antibiotic sources remains a global challenge, engaging research institutions, pharmaceutical companies, and academia (Kalpashree and Raveesh, 2013). In this context, endophytes are promising due to their capacity to produce unique bioactive metabolites. Among all extracts, *A. versicolor* (TE2) showed the strongest antibacterial and antifungal activity, particularly against *Bacillus subtilis*, outperforming gentamicin, in line with earlier studies (Li *et al.,* 2019; Wang *et al*., 2021). The unidentified isolate TE7 also exhibited potent antibacterial effects, comparable to *A. versicolor* (TE2). TE8 displayed consistent antibacterial activity but limited antifungal effects. These findings underscore the potential of unidentified isolates as sources of novel antimicrobials (Strobel and Daisy, 2003; Kusari *et al.,* 2012). *Penicillium* spp.1and 2 (TE3 and TE9) showed moderate, selective activity, mainly against *Candida albicans* and *Penicillium chrysogenum*, while *A. micronesiensis* (TE4) demonstrated weak antibacterial but moderate antifungal activity. Overall, the results support *T. erecta* as a promising source of endophytic fungi with antimicrobial potential.

5. Conclusion

This study demonstrates that endophytic fungi isolated from *Thunbergia erecta* possess significant antimicrobial potential. Among the isolates, *Aspergillus versicolor* (TE2) exhibited the strongest antibacterial and antifungal activities, in some cases surpassing standard antibiotics such as Gentamicin and Fluconazole. These findings highlight the value of endophytic fungi from underexplored medicinal plants as promising sources of novel bioactive compounds. Further research focusing on the purification, structural elucidation, and mechanism of action of the active metabolites is essential to assess their therapeutic potential and suitability for pharmaceutical applications.

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Author contributions

Mangalagowri M.S: Conceptualization, Data curation, Formal analysis, Investigation, Methodology; Resources, Software, Visualization Roles, Writing-original draft, Review and editing. **K. Krishna:** The corresponding author; Conceptualization; Validation; Investigation, formal analysis; Supervision; Roles: Review and editing.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Conflict of interest

The authors declare no conflict of interest.

References

1) Ahmed AM, Ibrahim AM, Yahia R, et al (2023) Evaluation of the anti-infective potential of the seed endophytic fungi of Corchorus olitorius through metabolomics and molecular docking approach. BMC Microbiol 23:355.
2) Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). Current protocols in molecular biology. John Wiley & Sons.
3) Brantner, A., Pfeiffer, K. P., and Brantner, H. (1994). Applicability of diffusion methods required by the pharmacopoeias for testing antibacterial activity of natural compounds. Die Pharmazie, 49(7), 512-516.
4) Barnett, H. L., & Hunter, B. B. (1998). Illustrated genera of imperfect fungi (4th ed.). APS Press.
5) Bhat M, Chakraborty B, Kumar RS, et al (2021) Biogenic synthesis, characterization and antimicrobial activity of Ixora brachypoda (DC) leaf extract mediated silver nanoparticles. Journal of King Saud University - Science 33:101296.
6) Chatterjee S, Sultana KW, Roy A, Chandra I (2015) An Overview of Ethnopharmacological and Phytochemical properties of Thunbergia. Open Access Journal of Medicinal and Aromatic Plants.

4(217), 2167-0412.

7) Giang, T. T. H., Quan, N. D., Linh, D. A., Lan, N. N., Huy, L. Q., Lien, N. T. K., & Hoang, N. H. (2021). Isolation and identification of endophytic fungi from Catharanthus roseus and Scutallaria barbata. Academia Journal of Biology, 43(2), 1-10.
8) Hadadi Z, Nematzadeh GA, Ghahari S (2020) A study on the antioxidant and antimicrobial activities in the chloroformic and methanolic extracts of 6 important medicinal plants collected from North of Iran. BMC Chemistry 14:33.
9) Kalpashree, M. M., & Raveesha, K. A. (2013). Antibacterial activity of Cycas circinalis ovules-a naked seeded gymnosperm.
10) Kumar S, Aharwal RP, Singh D, et al (2023) Isolation and Chemical Structural Elucidation of Antibacterial Bioactive Compounds from Endophytic Fungal Strain Phoma sp. D1. Curr Pharmacol Rep 9:128–143.
11) Kusari, S., Hertweck, C., and Spiteller, M. (2012). Chemical ecology of endophytic fungi: Origins of secondary metabolites. Chemistry & Biology, 19(7), 792–798.
12) Kusari, S., Hertweck, C., and Spiteller, M. (2012). Chemical ecology of endophytic fungi: Origins of secondary metabolites. Chemistry & Biology, 19(7), 792–798.
13) Kumari P, Singh A, Singh DK, et al (2021) Isolation and purification of bioactive metabolites from an endophytic fungus Penicillium citrinum of Azadirachta indica. South African Journal of Botany 139:449–457.
14) Kusari, S., Hertweck, C., and Spiteller, M. (2012). Chemical ecology of endophytic fungi: Origins of secondary metabolites. Chemistry & Biology, 19(7), 792–798.
15) Li, Y., Sun, L., Cheng, K., Lou, H., and Zhou, X. (2019). Antibacterial polyketides from a marine-derived fungus Aspergillus versicolor. Marine Drugs, 17(10), 563.
16) Magaldi S, Mata-Essayag S, Hartung de Capriles C, et al (2004) Well diffusion for antifungal susceptibility testing. Int J Infect Dis 8:39–45.
17) Nisa S, Khan N, Shah W, et al (2020) Identification and Bioactivities of Two Endophytic Fungi Fusarium fujikuroi and Aspergillus tubingensis from Foliar Parts of Debregeasia salicifolia. Arab J Sci Eng 45:4477–4487.
18) Refaey MS, Abdelhamid RA, Elimam H, et al (2021) Bioactive constituents from Thunbergia erecta as potential anticholinesterase and anti-ageing agents: Experimental and in silico studies. Bioorganic Chemistry 108:104643.
19) Strobel, G., and Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews, 67(4), 491–502.
20) Schmeda-Hirschmann G, Hormazabal E, Astudillo L, et al (2005) Secondary metabolites from endophytic fungi isolated from the Chilean gymnosperm Prumnopitys andina (Lleuque). World J Microbiol Biotechnol 21:27–32.
21) Schulz B, Boyle C, Draeger S, et al (2002) Endophytic fungi: a source of novel biologically active secondary metabolites*. Mycological Research 106:996–1004.
22) Shady NH, Sobhy SK, Mostafa YA, et al (2023) Phytochemical analysis and anti-infective potential of fungal endophytes isolated from Nigella sativa seeds. BMC Microbiol 23:343.
23) Stierle A, Strobel G, Stierle D (1993) Taxol and Taxane Production by Taxomyces andreanae, an Endophytic Fungus of Pacific Yew. Science 260:214–216.

24) Strobel GA (2003) Endophytes as sources of bioactive products. Microbes and Infection 5:535–544.

25) Strobel, G., and Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews, 67(4), 491–502.

26) Schulz, B., Boyle, C., Draeger, S., Römmert, A. K., and Krohn, K. (2002). Endophytic fungi: a source of novel biologically active secondary metabolites. Mycological research, 106(9), 996-1004.

27) Tan X-M, Chen X-M, Wang C-L, et al (2012) Isolation and Identification of Endophytic Fungi in Roots of Nine Holcoglossum Plants (Orchidaceae) Collected from Yunnan, Guangxi, and Hainan Provinces of China. Curr Microbiol 64:140–147.

28) Wang Z, Wang Y, Zheng L, (2014) Isolation and characterization of an antifungal protein from Bacillus licheniformis HS10. Biochemical and Biophysical Research Communications 454:48–52.

29) Wang, Y., Yang, M. H., Wang, X. B., Li, T. X., & Kong, L. Y. (2014). Bioactive metabolites from the endophytic fungus Alternaria alternata. Fitoterapia, 99, 153-158.

30) Wang, C., Li, J., Zang, X., Wei, Y., and Wang, J. (2021). Bioactive secondary metabolites from marine-derived Aspergillus species. RSC Advances, 11(14), 9074–9090.

31) Watanabe, T. (2002). Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species (2nd ed.). CRC Press