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Antifungal potential of Endophytic fungi isolated from *Ampelocissus latifolia* (Roxb.) Planch.

Meenakshi Baher[1], Basanti Brar[1]*, Ashok Kumar[2] and Dr. Ajit Kumar[2]

[1]Research Scholar, School of Microbiology, Om Sterling Global University, Hisar, Haryana

[1]*Associate Professor, HOD, School of Applied Sciences, Om Sterling Global University, Hisar, Haryana

[2]Seminal Applied Sciences Pvt. Ltd., Jaipur, Rajasthan

[2]Director and Principal Scientific Officer, Seminal Applied Sciences Pvt. Ltd., Jaipur, Rajasthan

For correspondence- basantibrar@osgu.ac.in

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Abstract

The present study explores the isolation, identification, and antimicrobial potential of endophytic fungi associated with *Ampelocissus latifolia* (Roxb.) Planch., a medicinal plant valued for its bioactive compounds. Five distinct fungal isolates (AL01-01, AL01-04, AL02-01, AL02-02, and AL02-03) were successfully isolated from leaf tissues using Potato Dextrose Agar (PDA). Morphological and microscopic analyses identified one dominant isolate as belonging to the genus *Acremonium*. Biochemical characterization revealed its ability to synthesize indole-3-acetic acid (IAA), with production increasing in response to elevated tryptophan concentrations, suggesting a potential role in plant growth promotion. The antifungal efficacy of *A. latifolia* extract was evaluated against four pathogenic fungi—*Aspergillus niger*, *Candida albicans*, *Penicillium chrysogenum*, and *Fusarium oxysporum*—using the PDA well diffusion method. Results demonstrated a clear concentration-dependent inhibition, with maximum activity observed at 80 µl against *C. albicans* (16 mm) and *A. niger* (14 mm). These findings indicate that *A. latifolia* harbors endophytic fungi capable of producing bioactive metabolites with significant antimicrobial and plant growth-promoting potential, highlighting its value as a natural source of therapeutic compounds.

1. Introduction

The association between plants and fungi represents one of the most ancient and ecologically significant symbiotic relationships in terrestrial ecosystems. Fossil evidence indicates that both endophytic and mycorrhizal associations were established when early plants colonized land, highlighting the crucial role of fungi in plant adaptation to terrestrial life (Chadha et al., 2015; Rai et al., 2014; Anjum et al., 2019). In primitive environments characterized by nutrient limitation and abiotic stress, fungal partners enhanced mineral acquisition, improved water uptake, and strengthened plant stress tolerance, thereby facilitating plant survival and diversification. This long co-evolutionary history underscores the foundational importance of plant–fungus interactions in shaping present-day terrestrial biodiversity and ecosystem functioning.

Among the different forms of plant–fungus interactions, endophytic fungi have emerged as particularly significant. Endophytes are microorganisms that colonize internal plant tissues without causing visible disease symptoms (Ekor, 2014; Mengistu, 2020). They inhabit roots, stems, leaves, and reproductive organs, often forming stable and asymptomatic associations. In many cases, the interaction is mutualistic, where the host plant provides nutrients and a protected niche, while the fungus enhances host fitness (Baron and Rigobelo, 2021). Phylogenetic studies suggest that endophytism has evolved independently across diverse fungal lineages and is nearly universal among terrestrial plants (Koide, 2019). However, these associations are dynamic and context-dependent; depending on environmental conditions and host–fungus compatibility, endophytes may function as mutualists, commensals, saprotrophs, or even latent pathogens (Koide, 2019).

The ecological significance of endophytic fungi extends beyond simple colonization. They contribute to plant resilience by improving tolerance to biotic and abiotic stresses, including pathogen attack, herbivory, drought, salinity, and heavy metal exposure (Manzur et al., 2022). Endophytes may induce systemic resistance, modulate plant hormonal signaling, and enhance physiological performance such as nutrient assimilation and chlorophyll production (Abdalla and Matasyoh, 2014). Additionally, they produce antimicrobial and antioxidative metabolites that protect plants against environmental threats (Mattoo and Nonzom, 2021). These multifaceted roles position endophytic fungi as critical components of plant defense systems and sustainable agricultural strategies (Aamir et al., 2020).

In recent years, endophytic fungi derived from medicinal plants have attracted considerable attention due to their remarkable capacity to produce diverse secondary metabolites with therapeutic potential (Varghese et al., 2024). These metabolites include alkaloids, terpenoids, flavonoids, phenolics, peptides, and polyketides, many of which exhibit antimicrobial, antifungal, antiviral, anticancer, antioxidant, and antiparasitic activities (Tiwari and Bae, 2022; Fan et al., 2022). Some endophytes are capable of synthesizing compounds structurally similar or identical to those of their host plants, suggesting shared biosynthetic pathways or metabolic exchange (Wen et al., 2022). This property is particularly important in the context of sustainable drug discovery, as endophytes may serve as alternative biofactories for valuable plant-derived compounds, thereby reducing pressure on medicinal plant resources.

Medicinal plants, owing to their rich phytochemical profiles, represent promising reservoirs of bioactive endophytic communities. Studies have demonstrated that endophytes isolated from medicinal plants exhibit strong antimicrobial activity against clinically important pathogens such as *Staphylococcus aureus* and *Escherichia coli* (Kulkarni et al., 2021). Furthermore, meta-analytical studies suggest that fungal endophytes can reduce insect herbivory and act as natural plant protectants (Gange et al., 2019). Despite growing global interest, targeted research on endophytic fungi associated with specific medicinal plants remains limited, particularly in underexplored regions, thereby necessitating further systematic investigations (Collinge et al., 2022).

Ampelocissus latifolia (Roxb.) Planch., commonly known as wild grape, belongs to the family Vitaceae and is widely distributed in tropical regions, with the Indian subcontinent recognized as its native habitat (Chaudri et al., 2015; Chaudhuri and Ray, 2015). Traditionally, this plant has been used for the treatment of bone fractures, dysentery, tuberculosis, and inflammatory conditions (Anand and Patni, 2016). Its therapeutic efficacy is attributed to the presence of bioactive compounds exhibiting anti-inflammatory, antimicrobial, and antioxidant properties. Leaf extracts rich in polyphenols have demonstrated phytotoxic and cytogenotoxic effects (Ray, 2014), and the plant has also been utilized as a natural dye source (Anand et al., 2018). However, certain reports caution about potential toxicity, emphasizing the importance of scientific validation and controlled application (Ray, 2014).

Morphologically, *A. latifolia* is characterized as a large herbaceous climber with hollow stems and reddish-brown grape-like fruits (Anand and Patni, 2016; Pednekar et al., 2013). Such herbaceous medicinal plants often provide favorable ecological niches for diverse endophytic fungal communities. Considering its medicinal value and rich phytochemical composition, *A. latifolia* represents a promising candidate for endophytic exploration. Investigating its associated endophytic fungi may uncover novel bioactive metabolites with antimicrobial and plant growth-promoting potential.

Therefore, this study is based on the following aspects: isolation and identification of endophytic fungi from the leaves of *Ampelocissus latifolia*; evaluation of their biochemical characteristics, particularly indole-3-acetic acid production; and assessment of their antifungal activity against selected pathogenic fungi. Through this investigation, the study seeks to contribute to the understanding of plant–endophyte interactions and to highlight the potential of medicinal plant-associated endophytes as valuable resources for sustainable agriculture and antimicrobial drug discovery.

2. Materials and methods

2.1 Endophytic fungi isolation from leaves of *Ampelocissus latifolia*

(a) Sample collection: Collection of fresh leaves of *Ampelocissus latifolia* was done from Jaipur region (Rajasthan). Sterile polyethylene bags were used to store the samples and then were transported under aseptic conditions for subsequent isolation of endophytic fungi.

(b) Endophytic fungi isolation: Collected fresh healthy leaves were processed for endophytic fungi isolation following **Araújo *et al.* (2002)**. Leaves were washed, surface-sterilized (75% ethanol, 5% sodium hypochlorite, and sterile water), and ~1 cm segments were placed on PDA with gentamycin to inhibit bacteria. Plates were incubated at 25–28°C for 15–20 days, and fungal growth was sub cultured for purification. PDA slants stored at 4°C were used to preserve the pure cultures.

2.2 Morphological and Biochemical identification of fungal endophytes

(a) Microscopy

To prepare a fungal specimen for microscopic observation, first clean a glass slide and add 1–2 drops of lactophenol cotton blue stain, which both kills and stains the fungus. Using a sterile needle or scalpel, transfer a small portion of an actively growing fungal colony onto the stain and gently tease it apart. Place a cover slip at an angle to avoid air bubbles and press lightly to spread the sample. Examine the slide under a microscope, starting with low power (10×) to locate structures, and then use higher magnifications (40× or 100×) to observe details such as hyphae, spores, and conidiophores **(Barnett and Hunter, 1998).**

(b) IAA (Indole Acetic Acid) Test

Indole-3-acetic acid production by endophytic fungi was evaluated using a modified version of the method described by **Gordon and Weber (1951)** by **Patten and Glick (2002)**. Fungal discs (5 mm) were inoculated into PDB containing 10% tartaric acid and varying tryptophan levels (0, 1, 2, 4 mg/mL), then incubated at 25 ± 1°C for 7 days in the dark at 120 rpm. After centrifugation (12,000 rpm, 10 min, 4°C), 2 mL Salkowski reagent was mixed with 1 mL supernatant and incubated for 30 min. A yellow-to-pink color change indicated IAA, quantified at 530 nm against a standard curve.

2.3 Active metabolite (s) sequential extraction from culture filtrate:

In a mixture of chloroform: methanol (6:3) ratio solvent, the processed filtrates were extracted (**Liu *et al.,* 2007**) with separating funnel and under pressure using vacuum evaporator at 40^0 C, the solvent was evaporated (**Augustine *et al*., 2005**). Then DMSO was used to dissolve the residue and further evaluation was done for antifungal activity.

2.4 Antifungal activity

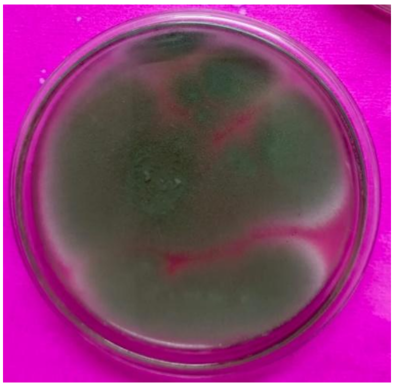
Antifungal efficacy of isolated endophytes has been carried out against various clinical important strains. Different strains have been collected for pursuing the activity. Clinically harvested and cultured *C. albicans* (ATCC No. 56766), *Fusarium oxysporum* (ATCC No. 48112), *Aspergillus niger* (ATCC No. 16888), and *Penicillium chrysogenum* (ATCC No. 9178) were isolated and sub cultured from the mother colonies.

The antifungal potency of the isolated flavonoids was evaluated in a similar manner, with slight modifications based on the method of **Hajieghrari *et al*., (2008).** The fungal strains were revived on Potato Dextrose Agar (PDA, Merck, Germany) and incubated at room temperature (25°C) for 1–2 days. The inoculums of the strains were mixed into autoclaved broth, and the final concentration was adjusted to approximately 10^6cells/mL. Petri dishes were dehydrated at 37°C for 15 minutes, after which wells were made in the autoclaved plates. After the test samples were added in the same manner as described above, the plates were incubated at 37°C. The same pattern of observations (in mm) was made after 1–2 days. The complete procedure was conducted in triplicate, and the mean values were determined.

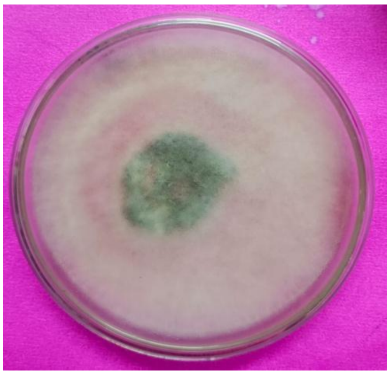
3. RESULTS

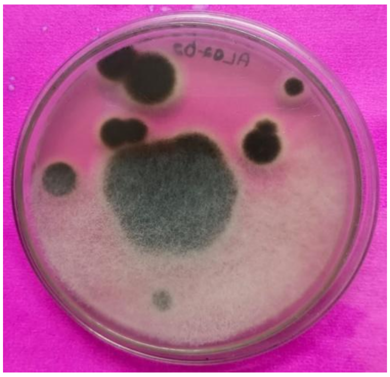
3.1 Isolation of Endophytic fungi from leaves of *Ampelocissus latifolia*

In the present study a total of 5 morphologically different fungal isolates (AL01-01, AL01-04, AL02-01, AL02-02, and AL02-03) were obtained from 150 leaf segments of experimental plants (fig.1).



(a) (b)





(c) **(d)**

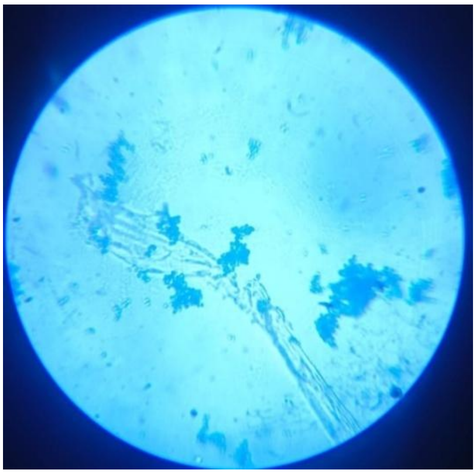
(e)

Figure 1: Isolation of endophytic fungi from *Ampelocissus latifolia* (a) AL01-01, (b) AL01-04, (c) AL02-01, (d) AL02-02, (e) AL02-03

3.2 Morphological and biochemical identification of endophytic fungi

(a) Microscopy

The endophytic fungal isolates obtained from the leaves of *Ampelocissus latifolia* were identified as *Penicillium* sp. (AL01-01), *Aspergillus niger* (AL01-04), *F. moniliforme* (AL02-01), *Alternaria* spp. (AL02-02) and *Acremonium strictum* (AL02-03) based on microscopic examination of their morphological characteristics (Figure 2).



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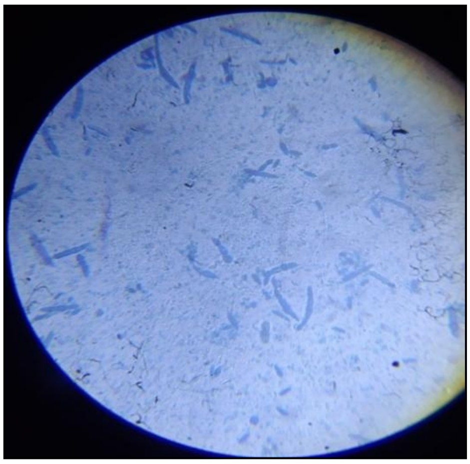

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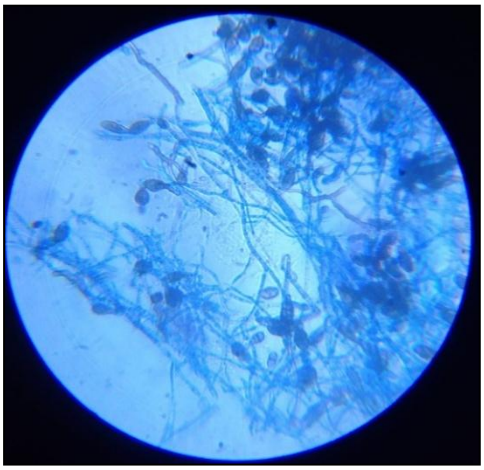

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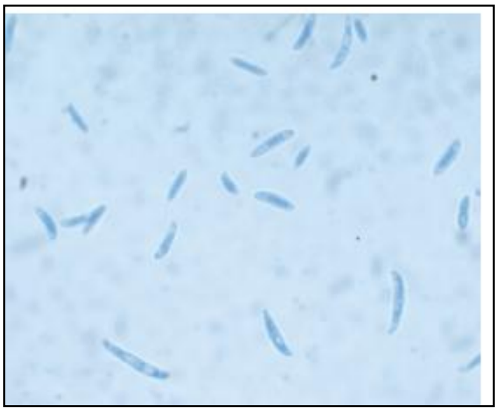


(e)

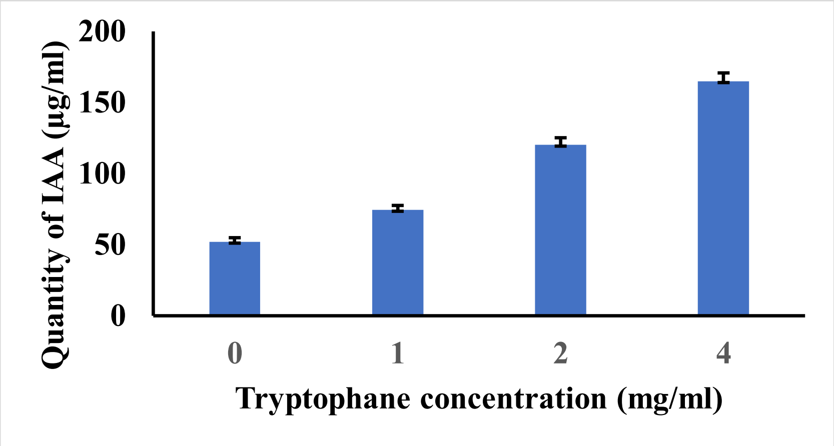
Figure 2: Microscopy of isolated endophytic fungi Strains (a) AL01-01, (b) AL01-04, (c) AL02-01, (d) AL02-02, (e) AL02-03

(b) IAA test

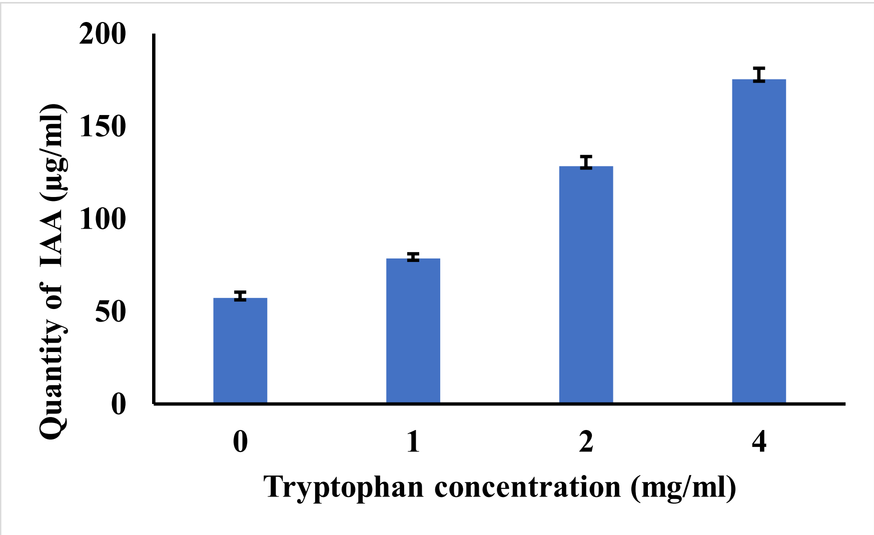
The quantification of indole-3-acetic acid (IAA) production by bacterial isolates at varying concentrations of tryptophan revealed a clear positive correlation between tryptophan availability and IAA synthesis. All five isolates (AL01-01, AL01-04, AL02-01, AL02-02, and AL02-03) produced measurable amounts of IAA even in the absence of tryptophan, indicating their inherent ability to synthesize IAA from endogenous precursors (Table 1). However, the addition of tryptophan markedly enhanced IAA production in all isolates. At 0 mg/ml tryptophan, IAA levels ranged between 52.10 and 57.20 µg/ml, while at 1 mg/ml the levels increased to between 72.80 and 78.60µg/ml. Further increments to 2 mg/ml and 4 mg/ml resulted in substantial rises, with maximum IAA production observed at 4 mg/ml, reaching up to 175.30µg/ml in isolate AL01-04. The overall trend demonstrates that IAA production increases proportionally with the concentration of tryptophan supplied. This pattern confirms that tryptophan serves as a key precursor for IAA biosynthesis, most likely through the indole-3-pyruvic acid pathway. The results suggest that supplementing tryptophan enhances the bacterial enzymatic conversion efficiency towards IAA formation. Among the isolates, AL01-04 consistently produced the highest IAA across all concentrations, indicating its superior IAA-producing capability.

Table 1: IAA Production Quantification

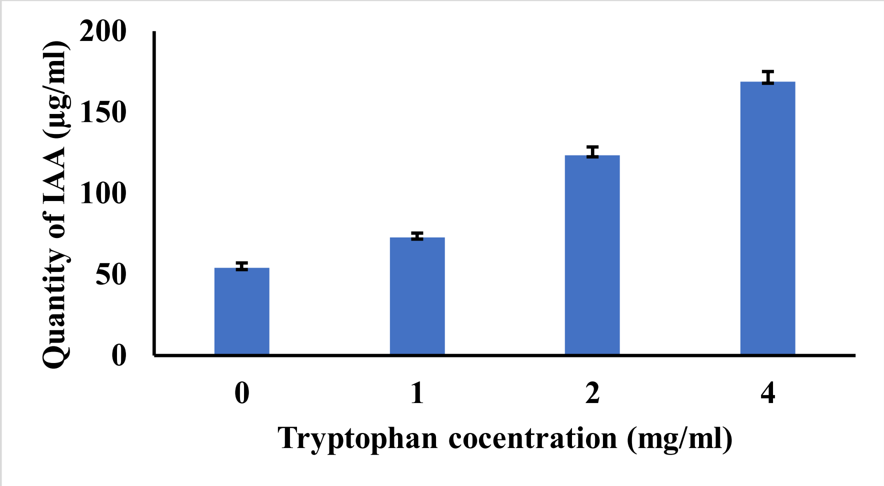
Tryptophan concentration (mg/ml)	Quantity of IAA (µg/ml)				
	AL01-01	AL01-04	AL02-01	AL02-02	AL02-03
0	52.10 ±2.80	57.20 ±3.20	54.00 ±3.10	56.10 ±2.90	55.27 ±3.00
1	74.50 ±3.10	78.60 ±2.50	72.80 ±2.70	75.90 ±2.80	76 ±2.6
2	120.30 ±4.90	128.40 ±5.20	123.50 ±5.10	127.20 ±5.00	125. ±5.00
4	165.00 ±5.80	175.30 ±6.00	168.90 ±6.20	172.40 ±6.10	170 ±6.12



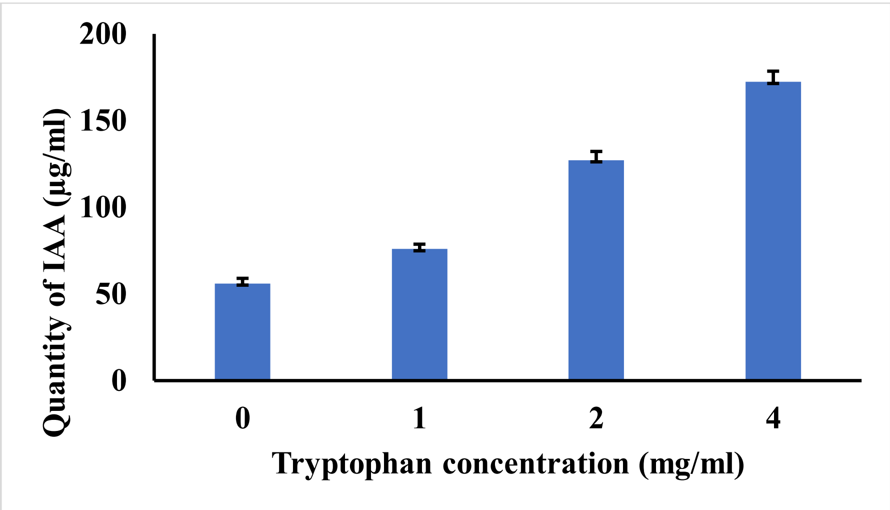
Graph 1: IAA Production Quantification of Endophytic Fungal strain- AL01-01



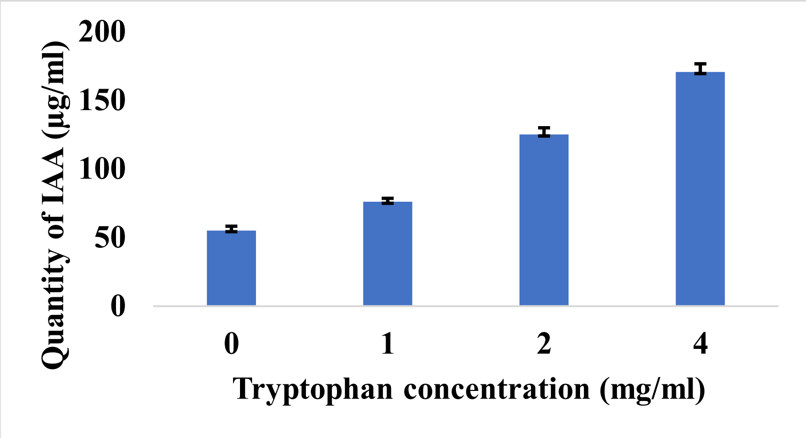
Graph 2: IAA Production Quantification of Endophytic Fungal strain- AL01-04



Graph 3: IAA Production Quantification of Endophytic Fungal strain- AL02-01



Graph 4: IAA Production Quantification of Endophytic Fungal strain- AL02-02



Graph 5: IAA Production Quantification of Endophytic Fungal strain- AL02-03

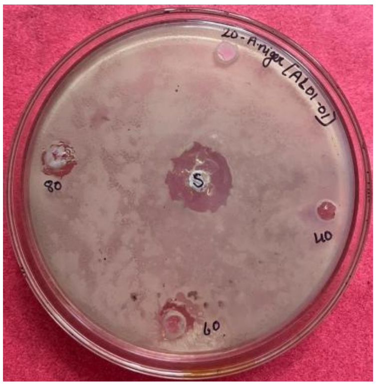
3.3 Antimicrobial activity

(a) Antifungal activity of *A. latifolia* isolated endophytic fungi against *A. niger*

The antifungal activity of *Ampelocissus latifolia* extract against *A. niger* showed that the extract was effective only at higher concentrations (Table 2). No zone of inhibition was observed at 20 µl and 40 µl, while moderate antifungal activity appeared at 60 µl and 80 µl, with inhibition zones ranging from 9 to 14 mm. The maximum inhibition (14 mm) was recorded for isolate AL02-02 at 80 µl. The standard antifungal showed a 20 mm zone, serving as a positive control (Figure 3). These results indicate that the antifungal potential of *A. latifolia* extract increases with concentration, suggesting the presence of active phytochemicals capable of suppressing *A. niger* growth.

Table 2: Antifungal activity of *A. latifolia* isolated endophytic fungi against *A. niger*

Sample name	Zone of inhibition (in mm)				
	Standard (20µl)	20µl	40µl	60µl	80µl
AL01-01	20	Nil	Nil	11	12
AL01-04	20	Nil	Nil	10	12
AL02-01	20	Nil	Nil	11	13
AL02-02	20	Nil	Nil	10	14
AL02-03	20	Nil	Nil	9	11



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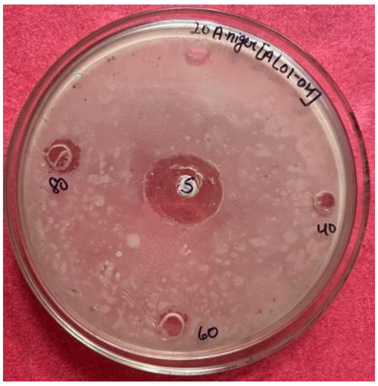

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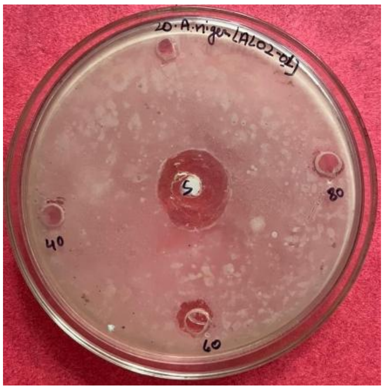

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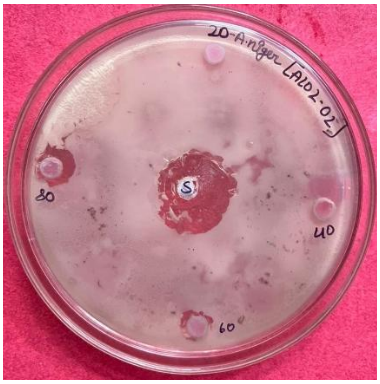

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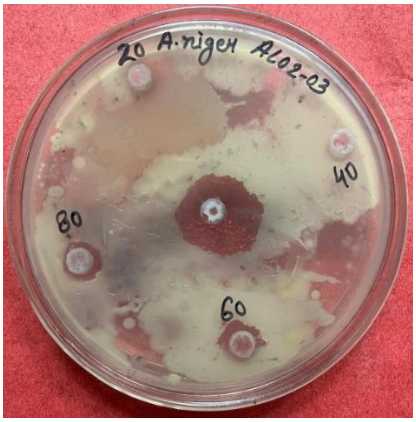


(e)

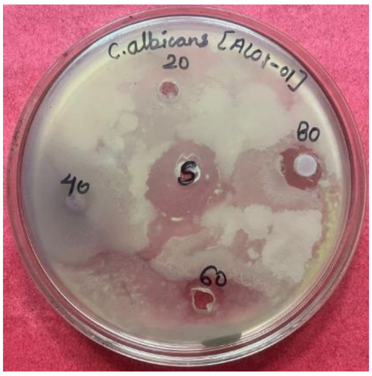
Figure 3: Antifungal activities against *A. niger* with (a) AL01-01, (b) AL01-04, (c) AL02-01, (d) AL02-02, (e) AL02-03

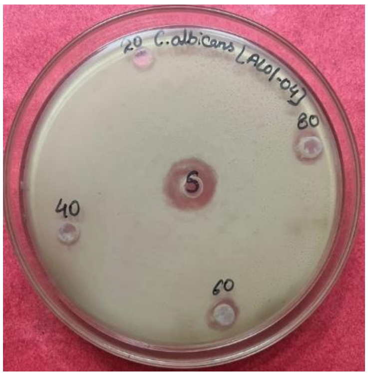
(b) Antifungal activity of *A. latifolia* isolated endophytic fungi against *C. albicans*

The antifungal activity of *A. latifolia* extract against *C. albicans* showed a concentration-dependent increase in inhibition (Table 3). No activity was observed at lower concentrations (20 µl and 40 µl), while moderate zones of inhibition appeared at higher concentrations (60 µl and 80 µl), ranging from 6 to 16 mm. The highest antifungal effect was recorded for isolate AL02-02, showing a 16 mm inhibition zone at 80 µl, followed by AL01-01 and AL02-01 (13 mm each). The standard antifungal (20 µl) exhibited a 24 mm inhibition zone, confirming assay effectiveness (Figure 4). These results indicate that *A. latifolia* extract possesses notable antifungal potential against *C. albicans*, particularly at higher concentrations, likely due to the presence of bioactive phytochemicals such as phenolic, flavonoids, and terpenoids that interfere with fungal cell wall integrity and growth.

Table 3: Antifungal activity of *A. latifolia* isolated endophytic fungi against *C. albicans*

Sample name	Zone of inhibition (in mm)				
	Standard (20µl)	20µl	40µl	60µl	80µl
AL01-01	24	Nil	Nil	9	13
AL01-04	24	Nil	Nil	9	11
AL02-01	24	Nil	Nil	10	13
AL02-02	24	Nil	Nil	10	16
AL02-03	24	Nil	Nil	6	9





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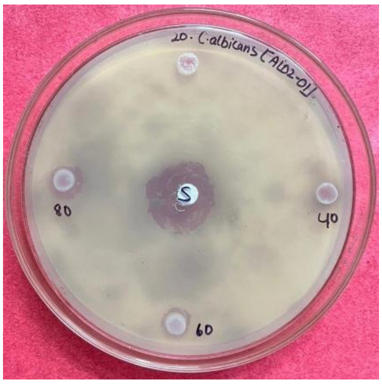


(c) **(d)**

(e)

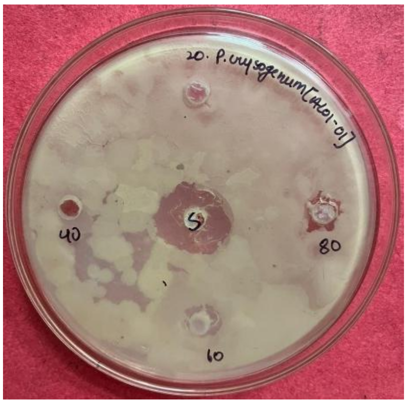
Figure 4: Antifungal activities against *C. albicans* with (a) AL01-01, (b) AL01-04, (c) AL02-01, (d) AL02-02, (e)AL02-03

(c) Antifungal activity of *A. latifolia* isolated endophytic fungi against *P. chrysogenum*

The antifungal activity of *A. latifolia* extract against *P. chrysogenum* showed a gradual increase with rising extract concentration (Table 4). No inhibition zones were observed at 20 µl, while mild to moderate antifungal activity appeared at 40 µl to 80 µl. The inhibition zones ranged from 8 to 15 mm, with the highest activity recorded for isolate AL01-04 (15 mm at 80 µl). The standard antifungal (20 µl) produced a 24 mm inhibition zone, confirming the reliability of the assay (Figure 5). These results indicate that *A. latifolia* extract exhibits dose-dependent antifungal activity against *P. chrysogenum*, suggesting that higher concentrations of the extract contain sufficient bioactive compounds—such as phenolics and flavonoids—that can inhibit fungal growth and cell wall development.

Table 4: Antifungal activity of *A. latifolia* isolated endophytic fungi against *P. chrysogenum*

Sample name	Zone of inhibition (in mm)				
	Standard (20µl)	20µl	40µl	60µl	80µl
AL01-01	24	Nil	Nil	10	12
AL01-04	24	Nil	10	12	15
AL02-01	24	Nil	Nil	12	13
AL02-02	24	Nil	Nil	12	8
AL02-03	24	Nil	Nil	10	12



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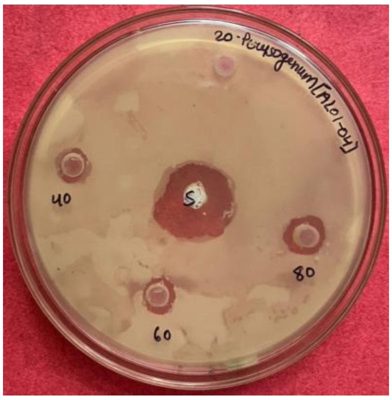

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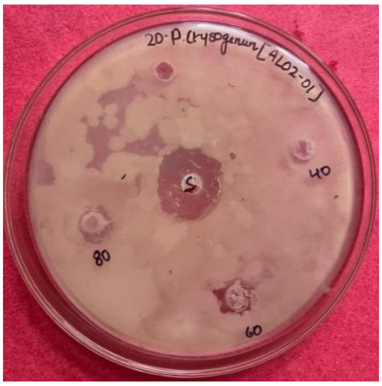

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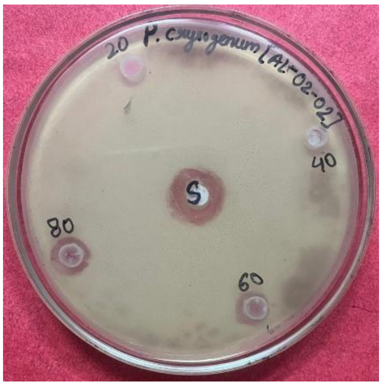

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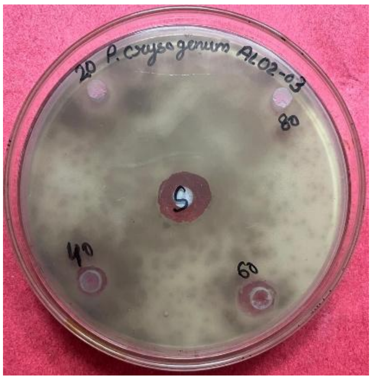


(e)

Figure 5: Antifungal activities against *P. chrysogenum* with (a) AL01-01, (b) AL01-04, (c) AL02-01, (d) AL02-02, (e)AL02-03

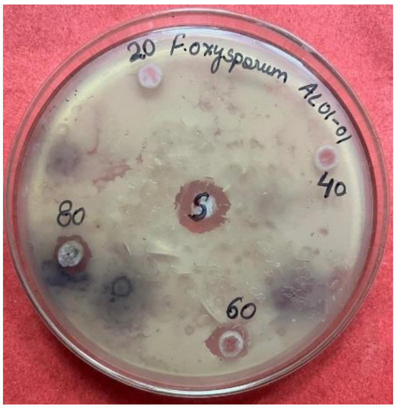
(d) **Antifungal activity of *A. latifolia* isolated endophytic fungi against *F. oxysporum***

No inhibition zones were observed at lower concentrations (20 µl and 40 µl) for most isolates, except AL02-01 and AL02-02, which showed mild activity (Table 5). As the concentration increased to 60 µl and 80 µl, the antifungal effect became more prominent, with inhibition zones ranging from 9 to 14 mm. The highest activity was recorded for isolates AL01-01 and AL02-02, both exhibiting 14 mm zones at 80 µl. The standard antifungal (20 µl) produced a 20 mm inhibition zone, serving as a positive control (Figure 6). These findings indicate that *A. latifolia* extract possesses moderate antifungal activity against *F. oxysporum*, with greater effectiveness at higher concentrations, likely due to the presence of potent bioactive compounds such as phenolics and flavonoids that interfere with fungal cell wall integrity and growth.

Table 5: Antifungal activity of *A. latifolia* isolated endophytic fungi against *F. oxysporum*

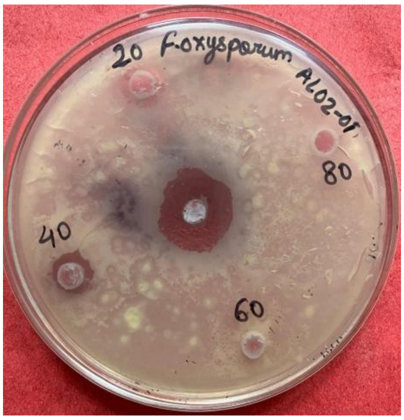
Sample name	**Zone of inhibition (in mm)**				
	Standard (20µl)	20µl	40µl	60µl	80µl
AL01-01	20	Nil	Nil	11	14
AL01-04	20	Nil	Nil	9	10
AL02-01	20	7	9	11	13

AL02-02	20	9	11	12	14
AL02-03	20	Nil	Nil	9	12

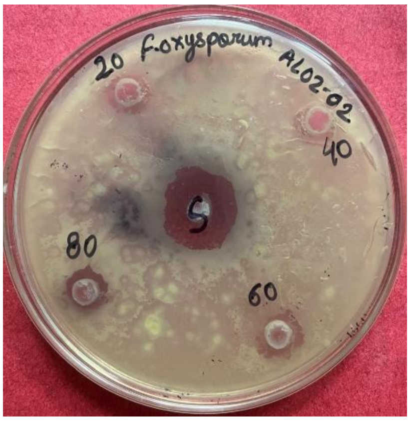


(a)

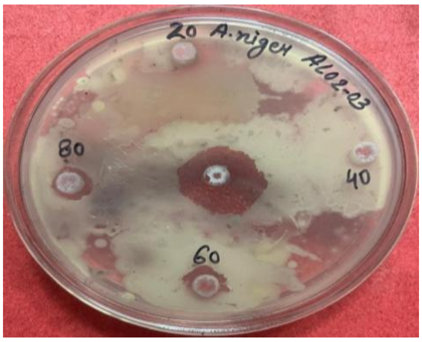
(b)



(c)



(d)



(e)

Figure 6: Antifungal activities against *F. oxysporum* with (a) AL01-01, (b) AL01-04, (c) AL02-01, (d) AL02-02, (e) AL02-03

4. Discussion

Endophytic fungi represent a functionally diverse and ecologically significant group of microorganisms that establish intimate associations with plant hosts. Despite increasing research interest, the physiological roles of endophytes and their complex communication mechanisms with host plants and co-existing microbial communities remain incompletely understood (Strobel, 2018; Suryanarayanan, 2013). The present study contributes to this expanding body of knowledge by investigating the diversity, plant growth-promoting ability, and antifungal potential of endophytic fungi isolated from *Ampelocissus latifolia*.

Five morphologically distinct endophytic fungal isolates were recovered from leaf tissues and identified as *Penicillium* sp., *Aspergillus niger, Fusarium moniliforme, Alternaria spp*., and *Acremonium strictum*. The occurrence of genera such as *Penicillium*, *Aspergillus*, *Fusarium*, and *Alternaria* among endophytes is consistent with earlier reports indicating that these taxa are common colonizers of internal plant tissues. Their widespread distribution suggests strong adaptive capabilities that allow them to persist as asymptomatic inhabitants within diverse host species.

Comparative studies further support these findings. Hussein et al. (2024) reported diverse endophytic communities in *Oxalis latifolia*, while Muñoz-Guerrero et al. (2021) documented 138 isolates from citrus tissues, highlighting tissue-specific colonization patterns. The relatively moderate number of isolates obtained in the present study may reflect host specificity, environmental factors, geographic location (Jaipur region), and the selective efficiency of surface sterilization techniques. Environmental conditions such as temperature, humidity, and host physiology are known to influence endophytic diversity and colonization frequency.

Importantly, the isolation of *Acremonium strictum* is noteworthy because species of this genus are frequently associated with bioactive metabolite production, including antimicrobial peptides and secondary metabolites with pharmaceutical relevance. Their presence within *A. latifolia* strengthens the hypothesis that medicinal plants often harbor metabolically versatile endophytes capable of producing biologically active compounds.

The quantification of indole-3-acetic acid (IAA) production revealed a clear positive correlation between tryptophan concentration and IAA synthesis across all isolates. The ability of isolates to produce measurable IAA even in the absence of exogenous tryptophan suggests endogenous biosynthetic pathways. However, supplementation with tryptophan significantly enhanced production, confirming its role as a precursor molecule in auxin biosynthesis, likely via the indole-3-pyruvic acid pathway.

Among the isolates, *Aspergillus niger* (AL01-04) demonstrated the highest IAA production (175.30 µg/mL at 4 mg/mL tryptophan), indicating superior plant growth-promoting potential. Comparable findings were reported by Mehmood et al. (2019), where *Aspergillus awamori* isolated from *Withania somnifera* produced significant levels of IAA under optimized culture conditions. Similarly, Munir et al. (2021) observed IAA production by *Curvularia lunata* and *Trichoderma* species, linking auxin production to enhanced root development and stress tolerance.

IAA-producing endophytes contribute to host plant fitness by stimulating root elongation, increasing lateral root formation, improving nutrient acquisition, and modulating hormonal balance. In medicinal plants such as *A. latifolia*, such interactions may enhance biomass accumulation and secondary metabolite production. Therefore, the isolates identified in this study may not only possess antimicrobial properties but also agricultural significance as potential biofertilizers or plant growth-promoting agents.

The antifungal assays demonstrated a clear concentration-dependent inhibitory effect of *A. latifolia*-derived extracts against *Aspergillus niger, Candida albicans, Penicillium chrysogenum*, and *Fusarium oxysporum*. No inhibition zones were observed at lower concentrations (20–40 µl), whereas significant inhibition appeared at 60–80 µl. This dose-responsive pattern indicates that bioactive compounds are present in sufficient quantities only at higher extract concentrations.

Among the isolates, AL02-02 (*Alternaria* spp.) consistently exhibited strong antifungal activity, particularly against *Candida albicans* (16 mm zone at 80 µl) and *Fusarium oxysporum* (14 mm). These findings suggest that metabolites produced either by the plant or its associated endophytes possess antifungal properties capable of interfering with fungal cell wall synthesis, membrane integrity, or enzymatic processes. The moderate but consistent activity against *C. albicans* is especially significant, as this opportunistic pathogen is responsible for various clinical infections and demonstrates increasing antifungal resistance. The observed inhibition zones, though smaller than standard antifungals, indicate promising preliminary efficacy that warrants further purification and compound characterization. Previous studies support the antimicrobial potential of A. latifolia. Khushbu et al. (2025) reported strong antibacterial activity of its root extract against *Bacillus anthracis*, confirming the plant's broad-spectrum bioactivity. However, Daniels and Ibiyemi (2021) observed that not all plant extracts exhibit antifungal properties, emphasizing the importance of host-endophyte specificity in bioactive metabolite production.

5. Conclusion

This study confirms that *Ampelocissus latifolia* harbors a diverse community of endophytic fungi possessing significant plant growth-promoting and antifungal properties. Five distinct fungal isolates were successfully obtained from leaf tissues and identified as *Penicillium* sp., *Aspergillus niger, Fusarium moniliforme, Alternaria spp*., and *Acremonium strictum*, indicating that this medicinal plant provides a suitable ecological niche for metabolically versatile endophytes. All isolates demonstrated the capacity to synthesize indole-3-acetic acid (IAA), a key phytohormone involved in root development and overall plant growth regulation. The observed increase in IAA production with tryptophan supplementation suggests the involvement of tryptophan-dependent biosynthetic pathways and highlights the potential of these isolates to enhance host plant growth and nutrient uptake under natural conditions.

In addition to their growth-promoting traits, the fungal extracts exhibited clear concentration-dependent antifungal activity against clinically and agriculturally important pathogens, including *Candida albicans* and *Fusarium oxysporum*. The progressive increase in inhibition zones at higher extract concentrations indicates the presence of active secondary metabolites, possibly phenolics, flavonoids, terpenoids, or endophyte-derived bioactive compounds capable of disrupting fungal growth and cellular integrity. Together, these findings underscore the ecological importance of *A. latifolia*-associated endophytes in promoting plant health and defending against pathogens. Furthermore, the demonstrated dual functionality plant growth enhancement and antifungal activity highlight their promising applications in sustainable agriculture as bioinoculants and in pharmaceutical research as potential sources of novel antimicrobial agents.

6. References

1) Aamir, M., Rai, K. K., Zehra, A., Kumar, S., Yadav, M., Shukla, V., & Upadhyay, R. S. (2020). 12 - Fungal endophytes: Classification, diversity, ecological role, and their relevance in sustainable agriculture. In A. Kumar & V. K. Singh (Eds.), Microbial Endophytes (pp. 291–323). doi:10.1016/B978-0-12-818734-0.00012-7
2) Abdalla, M. A., and Matasyoh, J. C. (2014). Endophytes as producers of peptides: an overview about the recently discovered peptides from endophytic microbes. Nat. Prod. Bioprospect. 4, 257–270.
3) Anand D.C. and Patni, V. (2016) Ampelocissus latifolia (ROXB) Planch. -A traditional plant with enormous medicinal and economic importance. International Journal of Pharma and Bio Sciences 2016 July; 7(3): (P) 303 – 307.
4) Anand, D. C., Mishra, P., & Patni, V. (2018). Quantitative evaluation of plant metabolites and dye extraction from leaves of Ampelocissus latifolia (Roxb.) planch. J Pharmacogn and Phytochem7 (4), 2260-2264.
5) Anjum R, Afzal M, Baber R, Khan MAJ, Kanwal W, Sajid W,Raheel A. 2019. Endophytes: as potential biocontrol agent—review and future prospects. J Agric Sci. 11:113.
6) Araújo, W.L.; Lima, A.O.S.; Azevedo, J.L.; Marcon, J.; Kuklinsky, S.J.; Lacava, P.T. Manual: Isolamento de Microrganismos Endofíticos; Universidade de São Paulo: Piracicaba, Brazil, 2002
7) Augustine SK, Bhavsar SP, Kapadinis (2005). A non polyene antifungal antibiotic from Streptomyces albidoflavous .J. Bioscience32 (2): 210-211.
8) Barnett, H. L., & Hunter, B. B. (1998). *Illustrated genera of imperfect fungi* (4th ed.). St. Paul, MN: APS Press.
9) Baron, N. C., & Rigobelo, E. C. (2021). Endophytic fungi: a tool for plant growth promotion and sustainable agriculture. *Mycology*, *13*(1), 39–55. https://doi.org/10.1080/21501203.2021.1945699
10) Chadha N, Mishra M, Rajpal K, Bajaj R, Choudhary DK, Varma A.2015. An ecological role of fungal endophytes to ameliorateplants under biotic stress. Arch Microbiol. Sep; 197(7):869–881. doi:10.1007/s00203-015-1130-3.
11) Chaudhuri Anwesa and Ray Sanjib* Determination of effective Allelopathic

(inhibitory) extract fractions of Ampelocissus latifolia (Roxb.) Planch. Leaf European Journal of Experimental Biology, 2015, 5(8):1-7

12) Chaudri A., Kundu L. M., Dutta S., Chatterjee S., Goswami S., Roy G.C. and Ray S.* Allelopathic effects of aerial parts aqueous extract of Ampelocissus latifolia (ROXB.) Planch. In apical meristem cells, Asian Journal of Plant Science and Research, 2015, 5(3):11-16.

13) Collinge, D. B., Jensen, B., and Jørgensen, H. J. L. (2022).Fungal endophytes in plants and their relationship to plant disease.Curr.Opin.Microbiol.69, 102177.

14) Cruz, J. S., da Silva, C. A., and Hamerski, L. (2020). Natural products from endophytic fungi associated with Rubiaceae species. J. Fungi 6, 128.

15) Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177

16) Fan, Y. Q., Ma, Z. H., Zhang, Y., Wang, Y. F., Ding, Y. S., Wang, C., et al. (2022). Sulfur-containing compounds from endophytic fungi: sources, structures and bioactivities. J. Fungi 8, 628.

17) Gange, A. C., Koricheva, J., Currie, A. F., Jaber, L. R., & Vidal, S. (2019). Meta-analysis of the role of entomopathogenic and unspecialized fungal endophytes as plant bodyguards. *New Phytologist*, *223*(4), 2002-2010.

18) Gordon SA, Weber RP. Colorimetric Estimation of Indoleacetic Acid. Plant Physiol. 1951 ;26(1):192-5.

19) Hajieghrari, B., Shahidi, F., Shams-Ardakani, M., & Ghasemi, S. (2008). Antifungal activity of some flavonoids isolated from *Bupleurum falcatum* L. *Iranian Journal of Pharmaceutical Research*, 7(4), 271–276.

20) Koide, R. T. (2019). Endophytic Fungi. *Encyclopedia of Life Sciences*, 1–6. https://doi.org/10.1002/9780470015902.a0027206

21) Kulkarni, S. A., Sharma, J. M., & Bobade, A. (2021). Antimicrobial power of endophytes from medicinal plants and enzyme production. International Journal of Creative Research Thoughts, 9, 173-181.

22) Liu X, Dong M, Chen X, Jiang M, Xin LV, Yan G (2007).Antioxidant activity of Phenolic of an endophytic Xylaria Sp.from Ginko biloba. Food and Chem105: 548- 554.

23) Manzur, M. E., Garello, F. A., Omacini, M., Schnyder, H., Sutka, M. R., and

Garcia-Parisi, P. A. (2022). Endophytic fungi and drought tolerance: ecophysiological adjustment in shoot and root of an annual mesophytic host grass. Funct. Plant Biol. 49, 272–282.

24) Mattoo, A. J., and Nonzom, S. (2021). Endophytic fungi: understanding complex cross-talks. Symbiosis 83, 237–264.

25) Mengistu AA. Endophytes: Colonization, Behaviour, and Their Role in Defense Mechanism. Int J Microbiol. 2020;2020:6927219

26) Patten CL, Glick BR. Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. Appl Environ Microbiol. 2002 ;68(8):3795-801.

27) Pednekar, P. A., Kulkarni, V., Raman B. (2013) Estimation of variation in the Elemental contents of methanolic soxhlet leaf extract of Ampelocissus latifolia (ROXB) Planch. By ICP-AES technique. International Journal of Pharmacy and Pharmaceutical Sciences Vol 5, July 2013

28) Perez C. Antibiotic assay by agar-well diffusion method.Acta Biology Medicine Experimental. 1990;15:113–115.

29) Rai M, Rathod D, Agarkar G, Dar M, Brestic M, Pastore GM,Marostica MR. 2014. Fungal growth promotor endophytes:a pragmatic approach towards sustainable food andagriculture. Symbiosis. Feb; 62(2):63–79. doi:10.1007/s13199-014-0273-3.

30) Ray, A. (2014). EVALUATION OF PHYTOTOXIC AND CYTOGENOTOXIC POTENTIALS OF LEAF AQUEOUS EXTRACT OF AMPELOCISSUS LATIFOLIA (ROXB.) PLANCH. IN RELATION TO ITS TOTAL POLYPHENOL CONTENT. International journal of pharma and bio sciences.

31) Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores.Anal Biochem. 1987;160:47–56.

32) Tiwari P, Bae H. Endophytic Fungi: Key Insights, Emerging Prospects, and Challenges in Natural Product Drug Discovery. Microorganisms. 2022;10(2):360

33) Varghese, S., Jisha, M. S., Rajeshkumar, K. C., Gajbhiye, V., Alrefaei, A. F., & Jeewon, R. (2024). Endophytic fungi: A future prospect for breast cancer therapeutics and drug development. *Heliyon*, *10*(13), e33995. doi:10.1016/j.heliyon.2024.e33995

34) Wen J, Okyere SK, Wang S, Wang J, Xie L, Ran Y, et al. Endophytic Fungi: An Effective Alternative Source of

Plant-Derived Bioactive Compounds for Pharmacological Studies. J Fungi. 2022;8(2):205

35) Strobel, G. The emergence of endophytic microbes and their biological promise. J. Fungi 2018, 4, 57.

36) Suryanarayanan, T.S. Endophyte research: Going beyond isolation and metabolite documentation. Fungal Ecol. **2013**, 6, 561–568.

37) Munir, E., Yurnaliza, Y., Lutfia, A., & Hartanto, A. (2021, June). Antifungal activity and IAA production by endophytic fungi isolated from Elettaria sp. In *IOP Conference Series: Earth and Environmental Science* (Vol. 782, No. 4, p. 042037). IOP Publishing.

38) Mehmood, A., Hussain, A., Irshad, M., Hamayun, M., Iqbal, A., & Khan, N. (2019). In vitro production of IAA by endophytic fungus

39) Hussein, J. M., Myovela, H., & Tibuhwa, D. D. (2024). Diversity of endophytic fungi from medicinal plant Oxalis latifolia and their antimicrobial potential against selected human pathogens. *Saudi Journal of Biological Sciences*, *31*(4), 103958.

40) Muñoz-Guerrero, J., Guerra-Sierra, B. E., & Alvarez, J. C. (2021). Fungal endophytes of Tahiti lime (Citrus citrus× latifolia) and their potential for control of Colletotrichum acutatum JH Simmonds causing anthracnose. *Frontiers in Bioengineering and Biotechnology*, *9*, 650351.

41) Khushbu, K., Rajak, K. K., Sonika, S., Mishra, S., & Verma, S. (2025). The antibacterial properties of Ampelocissus latifolia root extract against Bacillus anthracis. *BMC Complementary Medicine and Therapies*, *25*(1), 263.

42) Daniels, A. O., & Ibiyemi, D. A. (2021). Identification of the Functional Groups, Radical Scavenging Ability and Antimicrobial Properties of the Extract of Cleistopholis patens. *Funksec here*, *3*(1), 105-115.