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GC-MS/MS-Based metabolite profiling and bioactivity studies of endophytic fungi from the medicinal plant *Alternanthera philoxeroides* (mart.) Griseb

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Abstract

Endophytic fungi have garnered significant scholarly interest among researchers worldwide over the past three decades, attributed to their identification as a boundless reservoir of structurally and biologically innovative compounds. This study sought to analyze the traits of endophytic fungi., alongside biological assessments such as antimicrobial and antioxidant assay, derived from the medicinal plant *Alternanthera philoxeroides* (Mart.) Griseb. Six distinct endophytic fungal strains were successfully isolated and characterized based on morphological criteria and identified at the genus level, of which three were classified as *Carvularia* sp., while the remaining strains were identified as *Colletotrichum* sp., *Diaporthe* sp. and *Xylaria* sp. Numerous extracts of these endophytic fungi exhibited robust antibacterial properties and notable antioxidant activities. Chemical screening conducted via TLC and GC-MS/MS analysis revealed the presence of multiple potentially valuable secondary metabolites within the crude fungal extracts. The findings of the present study corroborate the advantageous effects of fungal endophytes, thereby position them as invaluable sources of bioactive compounds.

Keywords: *Alternanthera philoxeroides* (Mart.) Griseb.; Endophytic fungi; Morphology; antimicrobial; DPPH scavenging activity

Introduction

The nomenclature “endophyte” is etymologically rooted in the Greek terms “endon,” signifying within, and “phyton,” denoting plant (Wilson, 1995). Endophytes are microbial symbionts that inhabit the internal structures of the plant for the majority of their life cycle, typically without exerting adverse impacts on the host plant (Kandel *et al.* 2017) . Their ecological interactions are affected by multiple factors, including environmental conditions, host traits, the genetic diversity of microbial populations, and differences in nutrient exchange between the microorganism and the host (Mengistu, 2020). The isolation of microorganisms from particular plant species presents a significant opportunity to identify advantageous endophyte strains that synthesize host-specific secondary metabolites, which could be utilized in sustainable agriculture, pharmaceuticals, and various industrial applications (Sharma *et al.* 2021). These endophytes demonstrate the capacity to produce an extensive array of compounds that can substantially influence their host plants by promoting growth, offering protection against environmental stressors, enhancing overall fitness, fortifying resilience against abiotic and biotic challenges, and facilitating the accumulation of secondary metabolites (Jia *et al.* 2016; Nair and Padmavathy, 2014; Galindo-Solís and Fernández, 2022). Endophytes serve as a reservoir of numerous bioactive metabolites, including phenolic acids, alkaloids, quinones, steroids, saponins, tannins, xanthenes, anthraquinones, cytochalasins, benzofurans, lignans, polysaccharides, terpenoids, and plant growth hormones, thereby rendering them promising candidates for exhibiting antimalarial, antituberculosis, antidiabetic, anticancer anti-inflammatory, anti-arthritis, antiviral, and immunosuppressive properties, among others, and serving a vital source for drug discovery (Gouda *et al.* 2016; A. Sharma *et al.* 2020; Sibanda, 2017).

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Nature has been a wellspring of medicinal products for millennia, with numerous beneficial pharmaceuticals derived from plant sources (Sibanda, 2017). Continued investigations into medicinal plants for drug discovery are consistently producing novel and promising candidates addressing diverse pharmacological challenges, including antimicrobial resistance, cancer, HIV/AIDS, Alzheimer's disease, malaria, and pain management (Balunas and Kinghorn, 2005; Saha *et al.* 2022). Medicinal plants are recognized for their capacity to harbor potentially beneficial endophytic microorganisms, attributed to their bioactive compound profiles (Egamberdieva *et al.* 2017).

In contemporary scientific discourse, gas chromatography-mass spectrometry (GC–MS/MS) have been extensively employed for the elucidation of functional groups and the characterization of a variety of pharmacologically active compounds that are present within medicinal flora. Consequently, it is essential to evaluate the efficacy of endophytic fungi originating from traditional medicinal plants. Furthermore, the initial evaluation of biological and chemical properties affords an opportunity for additional insight into the identification of novel bioactive compounds.

Alternanthera philoxeroides, commonly referred to as Alligator weed, represents a herbaceous amphibious weed belonging to the Amaranthaceae family, with its origins traced back to South America. This species is predominantly located in stagnant and slowly moving aquatic environments, including creeks, channels, riverbanks, and adjacent regions that experience periodic flooding (Pan, 2007). Despite its South American origin, this weed has proliferated to numerous global locations. This species is characterized as a stoloniferous and rhizomatous perennial that exhibits rapid growth, typically forming a dense, interwoven mat (Sainty *et al.* 1997, X. Zhang *et al.* 2018). The entire plant is utilized as a vegetable for its health-promoting properties in Southeast Asia (Khamphukdee *et al.* 2021). In the local Bengali vernacular, this plant is designated as haicha shak, and the juice extracted from it is reputed to confer notable health benefits during episodes of fever, pain, and diabetes (Khatun *et al.* 2012). Documented pharmacological activities of this plant include anti-inflammatory and anti-arthritic effects (D. Sunmathi *et al.* 2016), antioxidant and anticoagulant properties (Khandker *et al.* 2022), antidepressant effects (Khamphukdee *et al.* 2018), antidementia capabilities (Khamphukdee *et al.* 2021), as well as antimicrobial (Sunmathi, 2016), antifungal (Amin *et al.* 2022) and antiviral (Zhang *et al.* 1988) activities. The initial phytochemical analysis of the methanolic extract revealed the existence of carbohydrates, proteins, amino acids, alkaloids, glycosides, steroids, flavonoids, tannins, and phenolic compounds (Sowjanya Pulipati and Puttagunta Srinivasa Babu, 2020, Pulipati *et al.* 2015). Previous studies reported several compounds from the endophytic fungi isolated from the plant *Alternanthera philoxeroides*, namely penichrysoamides A-C, penichrysoacid A and B, penichrysoamide D (Zhu *et al.* 2024); One new meroterpenoid-type alkaloid, oxalicine C, two new erythritol derivatives, penicierythritols A and B (Xu *et al.* 2020), Anhydrofusarubin, Fusarubin , Javanicin and 5-deoxybostrycoidin (Moni *et al.* 2022), neofusarubins A–D, fusofuranones A–C (Gao *et al.* 2024); 7-hydroxyldehydrocyclopeptin, 14,31-dimethoxy-penicopeptide A (Li *et al.* 2022); asperosperma A and asperosperma B (Ma *et al.* 2025); and 7,7'-di-O-demethyl-3,8'-bisiderin (Yi *et al.* 2024).

The diversity and dispersion of the fungal endophytes community are affected by various parameters including soil type, climate, environmental circumstances, tissue, genotype, age, location and plant host species (Osman *et al.* 2025). Given that *A. philoxeroides* is recognized as an invasive species exhibiting robust growth, the endophytic fungal community residing within the plant may potentially enhance the host's resilience to both abiotic and biotic stresses by synthesizing diverse bioactive secondary metabolites; furthermore, this plant remains largely uninvestigated concerning endophytes in Bangladesh, thereby prompting our interest in the isolation and assessment of potential endophytes that could yield novel bioactive compounds with a range of pharmacological properties.

The current study involves the extraction of fungal endophytes from the medicinal plant *Alternanthera philoxeroides* (Mart.) Griseb. within the geographical context of Bangladesh, utilizing a rigorously defined cultivation methodology, followed by the bioactivity assessment of fungal crude extracts through standardized protocols designed to evaluate antimicrobial and antioxidant activities, alongside chemical characterization through gas chromatography-mass spectrometry (GC-MS/MS) analysis.

Materials and methods

Collection, identification and extraction of plant material

In September 2023, fresh botanical specimens were collected from the Pabna district of Bangladesh, where these plants exhibit prolific growth. The plant was identified from Dhaka University Salar Khan Herbarium and a specimen representative has been archived there (Accession no. DUSH-10819). The air-dried botanical materials were subjected to drying at 40°C for 24 hours to diminish moisture levels. The desiccated specimens were subsequently pulverized and immersed in 100% methanol for 7 days at ambient temperature to facilitate the extraction of soluble constituents, followed by an additional 5 days of extraction (Rahaman *et al.* 2020).

Isolation of endophytic fungi

The plant specimens underwent an initial coarse washing with tap water to eliminate sand and residual debris. Subsequently, the plant parts were subjected to a rigorous surface sterilization protocol, which involved sequential immersion of the specimens in 70% ethanol (EtOH) followed by 10% sodium hypochlorite (NaOCl) solution, followed by immersion in EtOH (70%), with each solution exposure lasting between 1 to 2 minutes. Each plant component was then subjected to washes with sterile water three times to effectively clean any residual EtOH. The chosen samples were aseptically partitioned into smaller fragments (1–1.5 cm in length), which were subsequently placed onto culture media enriched with streptomycin sulfate (100 mg/L, to mitigate contamination of bacteria), and incubated in a dark environment at ambient temperature. A subset of positive controls (media containing unsterilized plant specimens) and a subset of negative controls (media devoid of any plant material) were also included to facilitate the detection of endophytic fungi and to evaluate the efficacy of the surface sterilization procedure. The preparation of the culture medium involved dissolving agar (18 g/L) in distilled water, followed by autoclaving at 121°C for 15 minutes. The apical regions of the hyphae that emerged from the initial cultures were subsequently transferred onto potato dextrose agar (PDA). Pure cultures of the isolated endophytic fungi were subsequently obtained utilizing serial dilution or streaking techniques (Noor *et al.* 2024).

Identification of endophytic fungi

Isolated endophytes were classified in accordance with morphological attributes, employing both macroscopic and microscopic criteria as delineated in recognized identification manuals (Hoque *et al.* 2022). In terms of macroscopic assessment, the distinct morphology of the fungal colonies (e.g., pigmentation, mycelial structure, hyphal characteristics, margin delineation, textural properties, growth velocity, etc.) was meticulously examined. For microscopic evaluation, slides were prepared from the cultures using the Lactophenol Cotton Blue (LPCB) staining method to enable detailed visualization of spore morphology (Shamly *et al.* 2014).

Preparation of fungal crude extracts

Each endophytic fungal isolate was cultured and maintained under dark conditions at 28 ± 2°C. Potato Dextrose Agar (PDA) was prepared at a concentration of 39 g/L in distilled water and subsequently sterilized by autoclaving at 121°C for 15 min (Hannana *et al.* 2020). After 21–28 days of incubation, once optimal mycelial growth was achieved, the culture media containing fungal metabolites were preserved by freezing at −20°C. (Hoshino *et al.* 2010). After thawing, the aqueous fraction of the fungal culture was separated from the mycelial biomass via filtration and subsequently subjected to chloroform extraction using a separating funnel. The fungal mycelia were then extracted with ethyl acetate (Jabeen *et al.* 2022). The organic fraction, represented by the ethyl acetate extract, was collected by filtration after a 7-day period, and the solvent was subsequently removed at 5-day intervals using a rotary evaporator maintained at 45°C (Chowdhury *et al.* 2020; Abd El Azeem *et al.* 2023).

Preliminary chemical screening

The crude extracts of *Alternanthera philoxeroides* (Mart.) Griseb. alongside its associated endophytic fungi underwent a screening process via thin-layer chromatography (TLC) for the preliminary elucidation of their chemical composition. Separations by TLC were conducted utilizing pre-coated silica gel 60, PF254, with a thickness of 0.2 mm on aluminium foil (Macherey-Nagel, Germany), employing a solvent mixture of 20% ethyl acetate in toluene. Spot detection was performed using a UV lamp (Analytik Jena US, USA) at 254 and 366 nm, followed by treatment with a 1% vanillin–sulfuric acid spray and heating at 110°C for 5 minutes (Mahmud *et al.* 2020; M. I. H. Khan *et al.* 2016).

Gas chromatography-mass spectrometry (GC–MS/MS) analysis

GC–MS/MS analyses of the fungal crude extract derived from the leaves and stems of *Alternanthera philoxeroides* (Mart.) Griseb were conducted utilizing the Perkin-Elmer Clarus 680 system (Perkin-Elmer, Inc., U.S.A.), which is equipped with a fused silica column, specifically a capillary column of Elite-5MS variety (30 m in length × 250 μ m in diameter × 0.25 μm in thickness). Pure helium gas (99.99%) served as the carrier gas, maintained at a constant flow rate of 1 mL/min. For the purpose of GC–MS/MS spectral detection, an electron ionization energy methodology was employed, characterized by a high ionization energy of 70 eV (electron Volts), with a scanning duration of 0.2 s and fragment ranges extending from 40 to 600 m/z. An injection volume of 1 μL was utilized (with a split ratio of 10:1), while the injector temperature was consistently maintained at 250°C. The temperature of the column oven was initially established at 50°C for a duration of 3 minutes, subsequently increased at a rate of 10°C

per minute to reach 280°C, followed by a final elevation to 300°C sustained for 10 minutes. The phytochemical constituents present within the test samples were identified through a comparative analysis of their retention time (in minutes), peak area, peak height, and mass spectral patterns against the spectral database of authentic compounds cataloged in the National Institute of Standards and Technology (NIST) library, employing a minimum match quality threshold of 80% as the evaluative criterion (Linstrom *et al.* 1997; Noor *et al.* 2025).

Biological activities of fungal crude extracts

Antimicrobial assay

The preliminary antimicrobial activity was assessed using the disc diffusion assay. Crude fungal extracts (2 mg) were dissolved in 200 µL of dichloromethane (DCM), and 10 µL of the resulting solution (corresponding to 100 µg per disc) was applied to each agar disc. The antimicrobial screening included two Gram-positive bacterial strains, *Staphylococcus aureus* (ATCC 9144) and *Bacillus megaterium* (ATCC 13578), and three Gram-negative bacterial strains, *Escherichia coli* (ATCC 11303), *Salmonella typhi* (ATCC 13311), and *Pseudomonas aeruginosa* (ATCC 27833), all obtained as pure cultures from the Institute of Food Science and Technology (IFST), BCSIR, Dhaka, Bangladesh. Furthermore, two fungal strains, *Aspergillus niger* (ATCC 1004) and *Aspergillus flavus* (UCFT 02), were procured from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). The growth inhibition zones around the discs were measured after incubating bacterial strains for 18 hours at 37°C and fungal strains for 48 hours at 28°C. The mean diameter of the inhibition zones (in mm) for each extract was compared with those of the reference standards, the antibacterial agent kanamycin (30 µg per disc) and the antifungal agent ketoconazole (30 µg per disc). Dichloromethane (DCM, 10 µl per disc) was included to evaluate any solvent-related effects on microbial growth. (Bauer *et al.* 1966, Lini *et al.* 2020).

Antioxidant activity

The antioxidant activity of the fungal crude extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, following the protocol established by Brand-Williams *et al.* (1995). The efficacy of each extract was compared with two standard antioxidants, ascorbic acid (vitamin C) and butylated hydroxyanisole (BHA). Crude extracts (1.6 mg) were dissolved in methanol (400 µL) and serially diluted to generate concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µg/mL. A 2 mL aliquot of each extract solution was mixed with an equal volume of DPPH solution in methanol (20 µg/mL) and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm, and the radical scavenging activity (%) was calculated using the standard formula. The IC_{50} value (µg/mL), defined as the concentration required to inhibit 50% of DPPH radicals, was determined for each extract as a measure of its antioxidant potential (Brand-Williams *et al.* 1995);

$$\text{Scavenging activity (\%)} = [(A_{Blank} - A_{Sample})/A_{Blank}] \times 100$$

Statistical analysis

The half-maximal inhibitory concentration (IC_{50}) for antioxidant activity was determined using logistic regression using MS excell. Calculations, graphs, tables, and figures were prepared using Microsoft Excel and Office software.

Results and discussion

Identification of endophytic fungi

A comprehensive total of six endophytic fungal strains, designated as APLE-1, APLE-2, APLE-3, APFE-4, APSE-1, and APSE-2, were systematically isolated and purified from the botanical specimen *Alternanthera philoxeroides* (Mart.) Griseb. These strains exhibited distinctive colony and microscopic morphological characteristics (refer to Table I and Table II) that may facilitate their differentiation. These fungal taxa were systematically classified into the genus level based on their macroscopic and microscopic morphological characteristics observed in the culture medium (illustrated in Figure 1-6 A-C). All of the six fungal strains were taxonomically classified up to the genus level based on their observable morphological traits. Specifically, for the strains APLE-2, APSE-1, and APSE-2, the conidiophores were characterized as brown, simple, or occasionally branched, with the conidia being dark brown; the terminal branched cells bore spores on newly developing sympodial growth points, comprising 3 to 5 cells that were more or less fusiform, typically exhibiting a bent or curved morphology, with one or two of the central cells enlarged, leading to their identification as *Carvularia* sp. The strain APLE-3 was classified as *Colletotrichum* sp., characterized by acervuli that were either disc-shaped or cushion-shaped, possessing a waxy texture, sub-epidermal positioning, and typically accompanied by dark spines or setae along the periphery or interspersed among the conidiophores; the conidiophores were identified as simple and elongated, while the conidia were hyaline, unicellular, and exhibited ovoid or oblong shapes. The strains APLE-1 was denoted as *Xylaria* sp. as it shows the characteristics of the circular, raised, and upper surface of the colony at first white and later turned into a brown to black color on PDA media

Table I. Microscopic characteristics of the isolated endophytes

Identified genus	Internal strain no.	Microscopically visible features		
		Mycelium	Conidia	Conidiophores
Xylaria sp.	APLE-1	Septate, hyaline and branched	Not seen	Not seen
Curvularia sp.	APLE-2	Septate, highly branched	Spores are oval and attached to conidiophores arising from a septate mycelium and are multicelled	Pigmented and geniculate
Colletotrichum sp.	APL3-3	Highly branched	Large, single-celled conidia with abundant sporulation rate	Short, simple, and dense
Diaporthe sp.	APLE-4	Septate, branched and hyaline	Not seen	Not found
Curvularia sp.	APSE-1	Septate and branched	Spores are oval and crescent-shaped and attached to conidiophores arising from a septate mycelium and are single or multicelled	Simple and geniculate
Curvularia sp.	APSE-2	Septate and branched	Spores are oval and attached to conidiophores arising from a septate mycelium and are multicelled	Simple or branched conidiophores are bent at the points where the conidia originate.

Table II. Macroscopic characteristics of isolated endophytes

Identified genus	Internal strain no.	Colony Morphology					
		Margin	Texture	Growth rate	Hyphae	Mycelia	Color of the reverse side
Xylaria sp.	APLE-1	Undulate	Wooly	Slow	Surficial	Cottony and dense near the center	White
Curvularia sp.	APLE-2	Filiform	Radial	Moderate	Aerial	Soft, dense, and aerial mycelium Soft, dense, and aerial mycelium	Reddish grey

Continued

Diaporthe sp.	APLE-4	Undulate	Wooly	Moderate	Surficial	Flat and rough not raised	Off white
Curvularia sp.	APSE-1	Entire	Radial	Fast	Surficial	Soft, dense and aerial mycelium	Black
Curvularia sp.	APSE-2	Filiform	Radial	Moderate	Aerial		Grayish Green

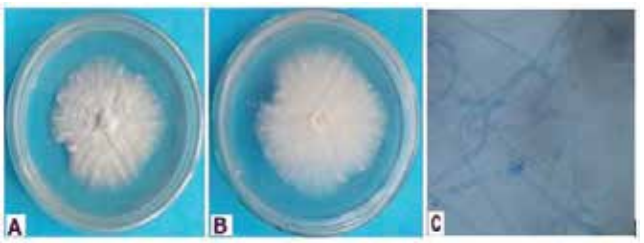

Fig. 1. Isolate APLE-1 (*Xylaria* sp.). (A) Surface of colony. (B) Reverse of colony. (C) Microscopic view

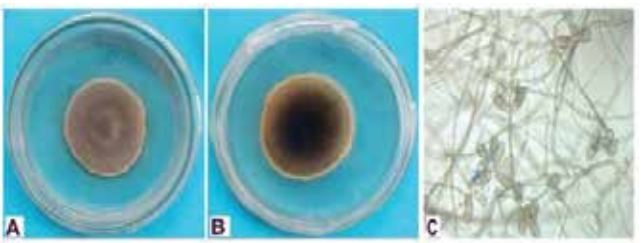

Fig. 2. Isolate APLE-2 (*Curvularia* sp.). (A) Surface of colony. (B) Reverse of colony. (C) Microscopic view

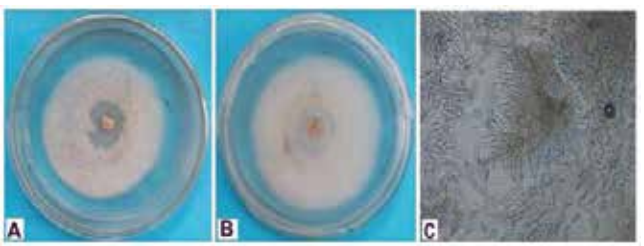

Fig. 3. Isolate APLE-3 (*Colletotrichum* sp.). (A) Surface of colony. (B) Reverse of colony. (C) Microscopic view

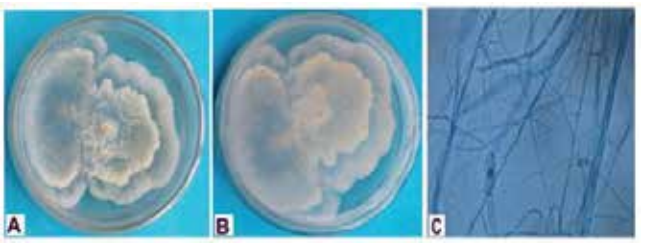

Fig. 4. Isolate APLE-4 (*Diaporthe* sp.). (A) Surface of colony. (B) Reverse of colony. (C) Microscopic view

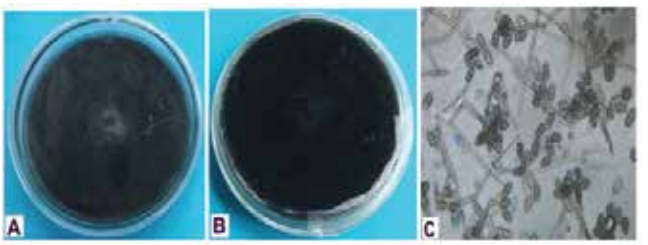

Fig. 5. Isolate APSE-1 (*Curvularia* sp.). (A) Surface of colony. (B) Reverse of colony. (C) Microscopic view

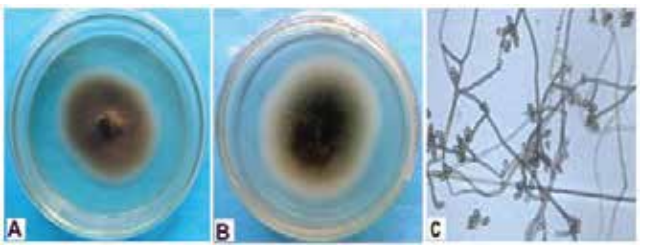

Fig. 6. Isolate APSE-2 (*Curvularia* sp.). (A) Surface of colony. (B) Reverse of colony. (C) Microscopic view

Table III. Antimicrobial activity of endophytic fungal strains isolated from *Alternanthera philoxeroides (Mart.) Griseb.* at a concentration of 100 µg/disc

Sample	Diameter of the inhibition zone (mm)[a]						
	B. megaterium	*S. aureus*	*P. aeruginosa*	*E. coli*	*S. typhi*	*A.niger*	*A.flavus*
APLE-1	8.5	9.5	-	-	9.5	-	-
APLE-2	18.5	26	29.5	17.5	17	-	-
APLE-3	6.5	-	7.5	7	-	-	-
APLE-4	17.5	16.5	25	15.5	16.5	-	-
APSE-1	-	-	-	-	-	-	-
APSE-2	-	-	-	-	-	-	-
Kanamycin(30 µg/disc)	30.5	29	33	32.5	29	nd	nd
Ketoconazole(30 µg/disc)	nd	nd	Nd	nd	nd	39	40
Solvent (Dichloromethane and Methanol)	-	-	-	-	-	-	-

[a] '–' indicates no activity detected and 'nd' indicates not done.

Table IV. Chemical screening of fungal extracts by thin-layer chromatography

Identified genus	Internal strain no.	Visual observation	Visibility under UV light (254 nm)	Visibility under UV light (366 nm)	Prospective compounds
Xylaria sp.	APLE-1	Brown	Dark green Light brown Blue Orange	Sky blue purple Yellowısh brown Light Purple Dark quenching	Flavonoids/Anthraquinone Coumarins/ Flavonoids Isocoumarıns Coumarins/ Flavonoids Anthraquinone Coumarins/ Anthocyanins
Curvularia sp.	APLE-2	Brown Orange Light brown Greenish yellow	Brown Dark green Green Light brown Green Light green Orange Sky blue	Bown Orange Orange Sky blue Sky blue Sky blue Sky blue Orange Dark quenching	Flavonoids Coumarins/ Flavonoids Isocoumarins Terpenoids/ Coumarins Flavonoids/Anthraquinone Coumarins Flavonoids/Anthraquinone Terpenoids Coumarins/ Anthocyanins
Colletotrichum sp.	APLE-3	Brown	Green Brown Brown Light Orange	Sky blue Purple Orange Purple	Flavonoids Coumarins Isocoumarins Terpenoids

Continued

			Sky blue	Sky blue	Flavonoids/ Coumarins
				Brown	Flavonoids/
				Purple	Anthraquinone
				Green	Coumarins
				Dark quenching	Flavonoids/ Coumarins
Diaporthe sp.	APLE-4	Brown	Orange	Red	Flavonoids/ Coumarins
		Light brown	Light green	Brown	Anthraquinone
		Yellow	Green	Red	Anthocyanins
			Orange	Red	Coumarins
				Orange	Isocoumarins
				Purple	Sterols
				Dark quenching	Anthocyanins
Curvularia sp.	APSE-1	Brown	Brown	Blue	Flavonoids
			Green	Blue	Coumarins
			Light green	Brown	Isocoumarins
				Dak quenching	Terpenoids
				Sky blue	Coumarins
				Sky blue	Coumarins/ Flavonoids
				Purple	-
				Dark quenching	Anthocyanins
Curvularia sp.	APSE-2	Brown	Green	Brown	Flavonoids
		Light brown	Light green	Green	Coumarins
				Orange	Isocoumarins
				Green	Terpenoids
				Dark quenching	Anthocyanins
				Green	Coumarins/ Flavonoids
				Green	Coumarins/ Flavonoids
				Dark quenching	Anthocyanins

and it was reported to develop finger-like stroma on the colony surface over time while APLE-4 is named as *Diaporthe* sp. as it displays the following criteria; The colony surface initially appears white, later developing a brownish-yellow pigmentation, accompanied by the formation of concentric rings with an undulate margin (Barnett and Hunter, 1960, Yan *et al.* 2018; Ramesh, 2014).

The current study elucidated the existence of six distinct endophytic fungal isolates from *Alternanthera philoxeroides* (Mart.) Griseb. The identification of these isolates from *Alternanthera philoxeroides* (Mart.) Griseb. was confirmed through precise microscopic features, supplemented by macroscopic (phenotypic) assessments. This constitutes the first documentation of fungal endophytes inhabiting *Alternanthera philoxeroides* (Mart.) Griseb. sourced from Bangladesh. Nevertheless, a limited number of studies regarding the diversity of endophytic fungi associated with the same species have been previously published. In a particular investigation, two hundred ninety-five fungal strains were extracted from *Alternanthera philoxeroides* (Mart.) Griseb. in China (CHEN and WANG 2017). The fungal strain Fusarium proliferatum, which is linked to foliar yellowing, was isolated from *Alternanthera philoxeroides* in China (Liu *et al.* 2018). Among the reported isolates, nine fungal endophytic strains, notably *Fusarium annulatum* and *F. solani,* were delineated from various segments of *A. philoxeroides*, including leaves, roots, and stems from a study in India (Biswas and Sarojini, 2024). In an additional investigation conducted in southern China, *Nimbya alternantherae* was extracted from *Alternanthera philoxeroides* (MeiMei, 2002). In a study, one hundred sixty-four fungal strains were successfully isolated from *Alternanthera philoxeroides*, among which two *Curvularia* and one *Colletotrichum* species existed (Zhou *et al.* 2015). A study reported *Cladosporium tenuissimum*; isolated from *Alternanthera philoxeroides* in India (Jayaram *et al.* 2023).

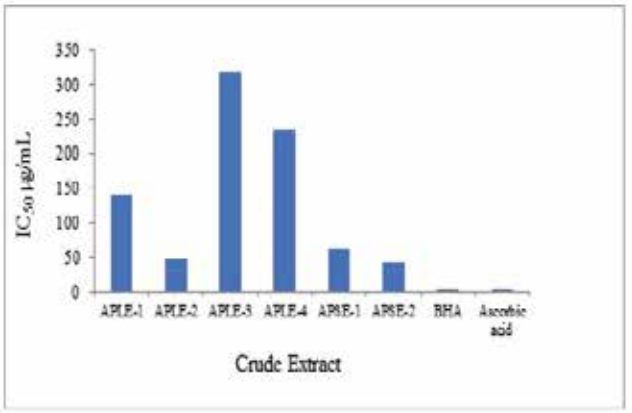

Fig. 7. Free radical scavenging activity of the isolated fungi

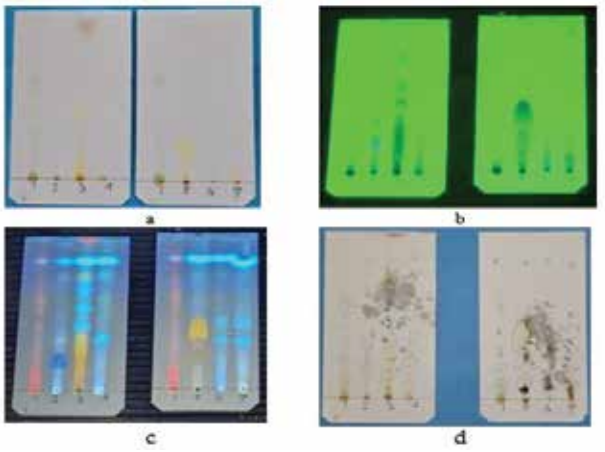

Fig. 8. TLC screening of the plant (1) and fungal extracts (2=APLE-1, 3=APLE-2, 4=APLE-3, 5=APLE-4, 6=APSE-1, 7=APSE-2) by visual observation (a), under UV at 254 nm (b), at 366 nm (c) and spraying with spray reagent (d)

Preliminary chemical screening

All fungal isolates were chemically profiled using thin-layer chromatography (TLC) to detect the presence of secondary metabolites. Each extract was analyzed by visual inspection under ultraviolet illumination at 254 and 366 nm, followed by treatment with vanillin–H_2SO_4 spray reagent for metabolite visualization (Table IV and Figure 8). The analysis of the TLC spots corresponding to the extracts revealed the presence of potentially prospective diverse secondary metabolites, including coumarins, isocoumarins, or their derivatives, flavonoids, steroids, terpenoids, anthocyanins, anthraquinones, or their derivatives (Harbome, 1998; Mahmud *et al.* 2020).

The analysis of TLC profile data derived from crude extracts has elucidated the presence of a variety of metabolites. The appearance of several bands on the TLC plate has signified the presence of substance classes including anthraquinones, naphthoquinones, and anthocyanins (Harbome, 1998; Chowdhury *et al.* 2017; N. Khan *et al.* 2018; Nasrin *et al.* 2021 and Noor *et al.* 2024), terpenoids, and steroids (Cohen *et al*. 2011, Ericsson and Ivonne, 2009), as well as flavonoids (Khalil *et al.* 2020), and isocoumarins (Krohn *et al.* 2004). The isolation of potential metabolites can be guided by the TLC profiles or characteristic NMR resonance peaks observed in the crude extracts. In this study, the different crude extracts displayed distinct TLC banding patterns, which may represent preliminary markers for bioactive metabolites pending further investigation.

Gas chromatography-mass spectrometry (GC-MS/MS) analysis

GC-MS/MS profiling of *Alternanthera philoxeroides* (Mart.) Griseb. (AP) and its associated endophytic fungal extracts—four derived from leaves (APLE-1, APLE-2,

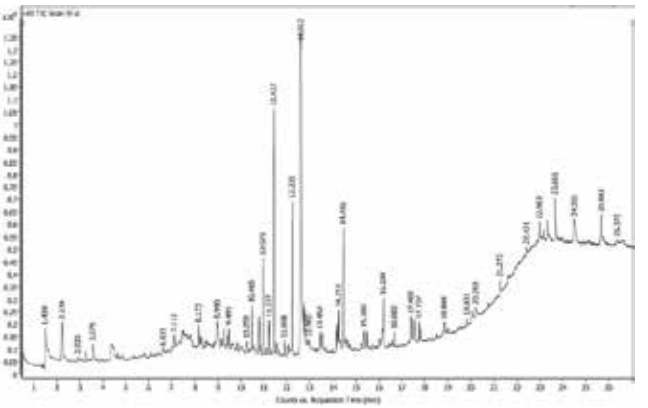

Fig. 9. GC-MS/MS chromatogram of the methanolic extract of AP

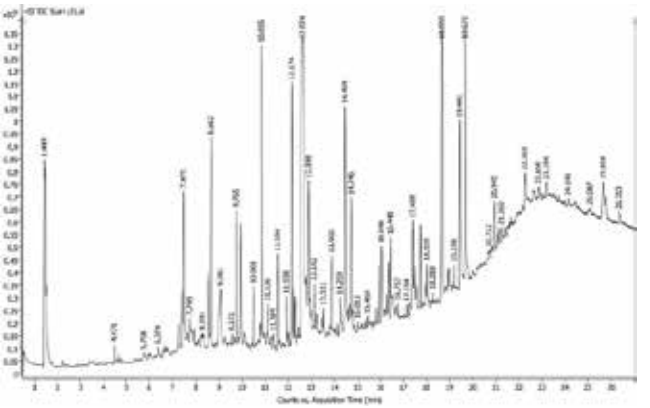

Fig. 10. GC-MS/MS chromatogram of the ethyl acetate fungal extract of APLE-1

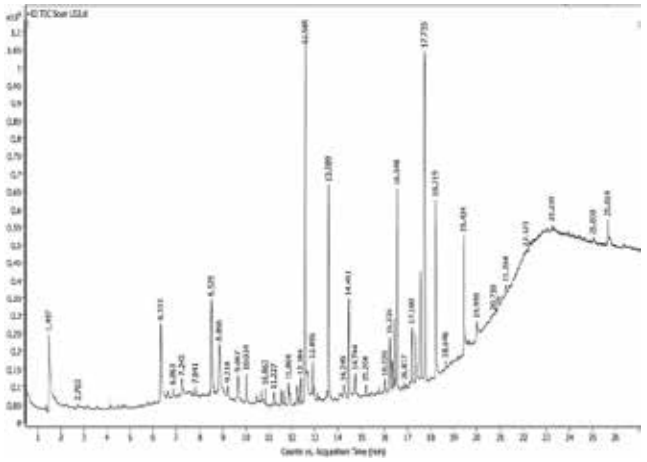

Fig. 11. GC-MS/MS chromatogram of the ethyl acetate fungal extract of APLE-2

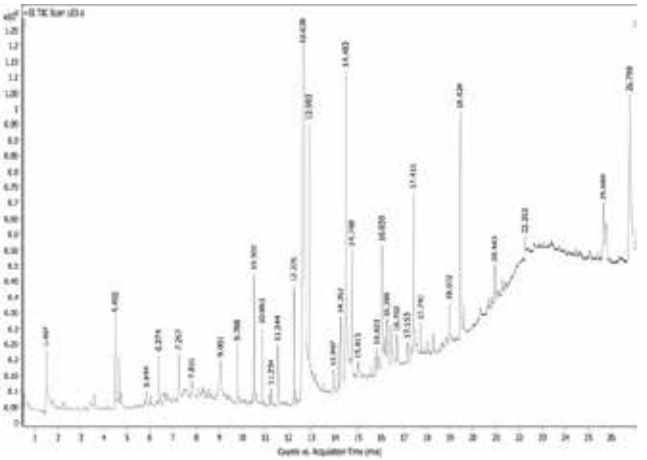

Fig. 12. GC-MS/MS chromatogram of the ethyl acetate fungal extract of APLE-3

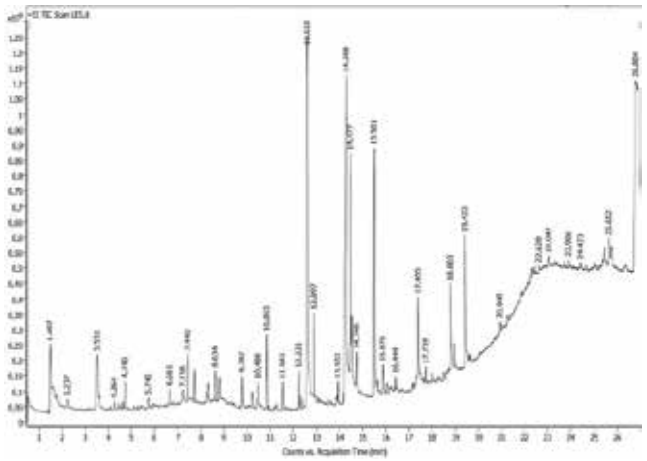

Fig. 13. GC-MS/MS chromatogram of the ethyl acetate fungal extract of APLE-4

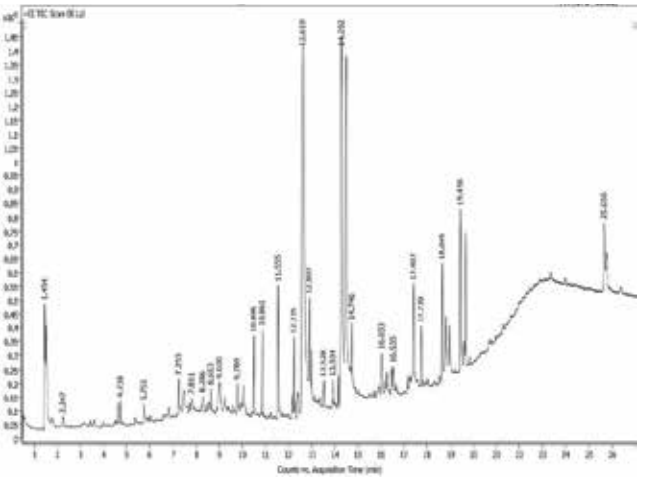

Fig. 14. GC-MS/MS chromatogram of the ethyl acetate fungal extract of APSE-1.

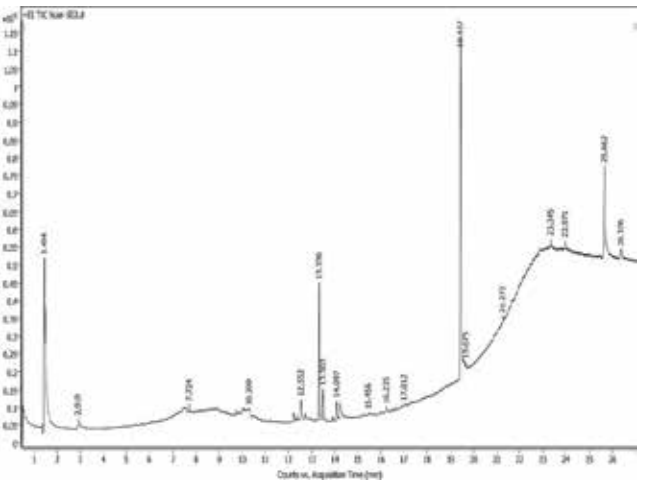

Fig. 15. GC-MS/MS chromatogram of the ethyl acetate fungal extract of APSE-2

APLE-3, APLE-4) and two from stems (APSE-1, APSE-2)—resulted in the identification of 12, 23, 11, 19, 17, 20, and 4 compounds, respectively. The distinct chromatographic patterns of these extracts are illustrated in Figures 9–15. Details of the identified metabolites, including retention time, molecular weight, molecular formula, and relative peak area, are summarized in Table V. Based on the percentage, the most prominent compounds were 13-Docosenamide, (Z)-acid (1) in the fungal extracts APSE-2 (26.40%), n-Hexadecanoic acid (2) in the plant and fungal extracts AP (25.26%) and APSE-1 (25.98%), APLE-3 (24.91%), APLE-4 (14.56%), APLE-2 (12.76%), and APLE-1 (9.36%) respectively, Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)- (3) in the fungal

Table V. Compounds identified in the medicinal plant *Alternanthera philoxeroides* (Mart.) Griseb. (AP) and its associated endophytic fungi, APLE-1, APLE-2, APLE-3, APLE-4, APSE-1, APSE-2 by GC-MS/MS analyses

Sample	Sl. no.	Retention time (min)	Name of the compounds	Molecular weight (g/mol)	Molecular formula	Peak area (%)
AP	1	1.50	.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	600.491	$C_{42}H_{64}O_2$	2.25
	2	7.51	Ethanone, 1-(3-hydroxy-4-methoxyphenyl)-	166.063	$C_9H_{10}O_3$	0.13
	3	8.17	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	180.115	$C_{11}H_{16}O_2$	0.65
	4	10.79	Loliolide	196.11	$C_{11}H_{16}O_3$	1.075
	5	12.24	Hexadecanoic acid, methyl ester	270.256	$C_{17}H_{34}O_2$	3.85
	6	12.61	n-Hexadecanoic acid	256.24	$C_{16}H_{32}O_2$	25.26
	7	14.15	Methyl stearate	298.287	$C_{19}H_{38}O_2$	0.51
	8	14.25	6-Octadecenoic acid	282.256	$C_{18}H_{34}O_2$	1.42
	9	14.45	Octadecanoic acid	284.272	$C_{18}H_{36}O_2$	3.62
	10	16.19	4,8,12,16-Tetramethylheptadecan-4-olide	324.303	$C_{21}H_{40}O_2$	0.95
	11	17.40	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330.277	$C_{19}H_{38}O_4$	0.75
	12	25.66	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-	470.376	$C_{31}H_{50}O_3$	2.60
APLE-1	1	4.47	Benzeneacetic acid	136.052	$C_8H_8O_2$	0.28
	2	6.66	Benzeneethanol, 4-hydroxy-	138.068	$C_8H_{10}O_2$	0.07
	3	7.47	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-	240.1	$C_{12}H_{16}O_5$	2.47
	4	10.50	Tetradecanoic acid	228.209	$C_{14}H_{28}O_2$	0.70
	5	11.55	Pentadecanoic acid	242.225	$C_{15}H_{30}O_2$	1.20
	6	12.24	Hexadecanoic acid, methyl ester	270.256	$C_{17}H_{34}O_2$	0.64

Continued

	7	12.47	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	210.137	$C_{11}H_{18}N_2O_2$	0.08
	8	12.62	n-Hexadecanoic acid	256.24	$C_{16}H_{32}O_2$	9.36
	9	13.53	Heptadecanoic acid	270.256	$C_{17}H_{34}O_2$	0.21
	10	13.90	4(15)-Selinene-11,12-diol	238.193	$C_{15}H_{26}O_2$	1.79
	11	14.15	Methyl stearate	298.287	$C_{19}H_{38}O_2$	0.14
	12	14.26	6-Octadecenoic acid	282.256	$C_{18}H_{34}O_2$	0.87
	13	14.47	Octadecanoic acid	284.272	$C_{18}H_{36}O_2$	3.96
	14	14.74	Bacteriochlorophyll-c-stearyl	840.54	$C_{52}H_{72}MgN_4O_4$	1.65
	15	16.45	17-Pentatriacontene	490.548	$C_{35}H_{70}$	0.89
	16	17.41	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330.277	$C_{19}H_{38}O_4$	1.01
	17	17.74	Bis(2-ethylhexyl) phthalate	390.277	$C_{24}H_{38}O_4$	1.00
	18	18.02	17-Pentatriacontene	490.548	$C_{35}H_{70}$	0.52
	19	18.66	Dechlorogriseofulvin	318.11	$C_{17}H_{18}O_6$	5.55
	20	19.20	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	390.277	$C_{24}H_{38}O_4$	0.32
	21	19.44	13-Docosenamide, (Z)-	337.334	$C_{22}H_{43}NO$	2.90
	22	19.67	Griseofulvin	352.071	$C_{17}H_{17}ClO_6$	5.81
	23	22.26	Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-	394.324	$C_{28}H_{42}O$	0.49
APLE-2	1	8.52	1-(4-tert-Butylphenyl)propan-2-one	190.136	$C_1H_{18}O$	3.83
	2	8.87	Gallacetophenone-4'-methylether	182.058	$C_9H_{10}O_4$	2.68
	3	12.29	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276.173	$C_{17}H_{24}O_3$	0.13

Continued

	4	12.38	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	210.137	$C_{11}H_{18}N_2O_2$	0.64
	5	12.58	n-Hexadecanoic acid	256.24	$C_{16}H_{32}O_2$	12.76
	6	13.59	Curvulin	238.084	$C_{12}H_{14}O_5$	5.15
	7	14.45	Octadecanoic acid	284.272	$C_{18}H_{36}O_2$	2.61
	8	16.22	9,10-Anthracenedione, 1,8-dihydroxy-3-methyl-	254.058	$C_{15}H_{10}O_4$	1.57
	9	17.73	Bis(2-ethylhexyl) phthalate	390.277	$C_{24}H_{38}O_4$	10.77
	10	19.43	13-Docosenamide, (Z)-	337.334	$C_{22}H_{43}NO$	3.52
	11	25.66	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-	470.376	$C_{31}H_{50}O_3$	1.18
APLE-3	1	1.50	2-(Hydroxymethyl)-2,4a,7,10b-tetramethyl-1a,2a,4,4a,10,10a,10b,11a-octahydro-2H,7H-[1]benzoxireno[3,4-f]pyrano[2,3-b]chromene-9,11(3H,8H)-dione, TMS	448.228	$C_{24}H36O_6Si$	2.12
	2	4.49	Benzeneacetic acid	136.052	$C_8H_8O_2$	1.94
	3	6.37	Benzeneacetamide	135.068	C_8H_9NO	0.92
	4	10.50	Tetradecanoic acid	228.209	$C_{14}H_{28}O_2$	1.77
	5	10.86	Bacteriochlorophyll-c-stearyl	840.54	$C_{52}H_{72}MgN_4O_4$	0.89
	6	12.23	Hexadecanoic acid, methyl ester	270.256	$C_{17}H_{34}O_2$	1.40
	7	12.30	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276.173	$C_{17}H_{24}O_3$	0.16
	8	12.63	n-Hexadecanoic acid	256.24	$C_{16}H_{32}O_2$	24.91
	9	12.90	Hexadecanoic acid, ethyl ester	284.272	$C_{18}H_{36}O_2$	4.26
	10	14.15	Heptadecanoic acid, 9-methyl-, methyl ester	298.287	$C_{19}H_{38}O_2$	0.63
	11	14.26	6-Octadecenoic acid	282.256	$C_{18}H_{34}O_2$	1.13

Continued

	12	14.48	Octadecanoic acid	284.272	$C_{18}H_{36}O_2$	8.37
	13	16.45	17-Pentatriacontene	490.548	$C_{35}H_{70}$	0.46
	14	17.41	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330.277	$C_{19}H_{38}O_4$	2.96
	15	17.74	Bis(2-ethylhexyl) phthalate	390.277	$C_{24}H_{38}O_4$	0.34
	16	19.44	13-Docosenamide, (Z)-	337.334	$C_{22}H_{43}NO$	4.20
	17	19.6	Sebacic acid, di(2-propylpentyl) ester	426.371	$C_{26}H_{50}O_4$	0.33
	18	22.26	Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-	394.324	$C_{28}H_{42}O$	0.28
	19	26.80	Cytochalasin H	493.283	$C_{30}H_{39}NO_5$	8.72
APLE-4	1	7.72	2,4-Di-tert-butylphenol	206.167	$C_{14}H_{22}O$	0.49
	2	10.86	Bacteriochlorophyll-c-stearyl	840.54	$C_{52}H_{72}MgN_4O_4$	1.10
	3	11.54	Pentadecanoic acid	242.225	$C_{15}H_{30}O_2$	0.47
	4	12.23	Hexadecanoic acid, methyl ester	270.256	$C_{17}H_{34}O_2$	0.59
	5	12.30	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276.173	$C_{17}H_{24}O_3$	0.20
	6	12.61	n-Hexadecanoic acid	256.24	$C_{16}H_{32}O_2$	14.54
	7	13.88	8,11-Octadecadienoic acid, methyl ester	294.256	$C_{19}H_{34}O_2$	0.13
	8	13.93	9-Octadecenoic acid, methyl ester, (E)-	296.272	$C_{19}H_{36}O_2$	0.34
	9	14.15	Methyl stearate	298.287	$C_{19}H_{38}O_2$	0.17
	10	14.29	6-Octadecenoic acid	282.256	$C_{18}H_{34}O_2$	11.91
	11	14.48	Octadecanoic acid	284.272	$C_{18}H_{36}O_2$	5.22
	12	14.54	Ethyl Oleate	310.287	$C_{20}H_{38}O_2$	1.08
	13	17.40	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330.277	$C_{19}H_{38}O_4$	1.48
	14	18.80	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	356.293	$C_{21}H_{40}O_4$	2.18

Continued

	15	19.43	13-Docosenamide, (Z)-	337.334	$C_{22}H_{43}NO$	2.79
	16	19.66	Griseofulvin	352.071	$C_{17}H_{17}ClO_6$	0.07
	17	26.80	Cytochalasin H	493.283	$C_{30}H_{39}NO_5$	10.15
APSE-1	1	10.49	Tetradecanoic acid	228.209	$C_{14}H_{28}O_2$	1.22
	2	10.86	Bacteriochlorophyll-c-stearyl	840.54	$C_{52}H_{72}MgN_4O_4$	1.43
	3	11.55	Pentadecanoic acid	242.225	$C_{15}H_{30}O_2$	2.19
	4	12.23	Hexadecanoic acid, methyl ester	270.256	$C_{17}H_{34}O_2$	1.15
	5	12.29	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276.173	$C_{17}H_{24}O_3$	0.30
	6	12.62	n-Hexadecanoic acid	256.24	$C_{16}H_{32}O_2$	25.98
	7	13.53	Heptadecanoic acid	270.256	$C_{17}H_{34}O_2$	0.47
	8	13.93	9-Octadecenoic acid, methyl ester, (E)-	296.272	$C_{19}H_{36}O_2$	0.47
	9	14.15	Methyl stearate	298.287	$C_{19}H_{38}O_2$	0.44
	10	14.29	6-Octadecenoic acid	282.256	$C_{18}H_{34}O_2$	12.06
	11	14.49	Octadecanoic acid	284.272	$C_{18}H_{36}O_2$	10.09
	12	16.45	17-Pentatriacontene	490.548	$C_{35}H_{70}$	0.40
	12	17.41	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330.277	$C_{19}H_{38}O_4$	1.57
	14	17.74	Bis(2-ethylhexyl) phthalate	390.277	$C_{24}H_{38}O_4$	0.84
	15	18.65	Dechlorogriseofulvin	318.11	$C_{17}H_{18}O_6$	1.77
	16	18.80	2,3-Dihydroxypropyl elaidate	356.293	$C_{21}H_{40}O_4$	0.92
	17	18.97	Octadecanoic acid, 2,3-dihydroxypropyl ester	358.308	$C_{21}H_{42}O_4$	0.59
	18	19.43	13-Docosenamide, (Z)-	337.334	$C_{22}H_{43}NO$	2.99
	19	19.66	Griseofulvin	352.071	$C_{17}H_{17}ClO_6$	2.06

Continued

	20	25.66	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-	470.376	$C_{31}H_{50}O_3$	2.26
APSE-2	1	12.55	n-Hexadecanoic acid	256.24	$C_{16}H_{32}O_2$	1.47
	2	14.25	2,3-Dihydroxypropyl elaidate	356.293	$C_{21}H_{40}O_4$	1.17
	3	19.44	13-Docosenamide, (Z)-	337.334	$C_{22}H43NO$	26.40
	4	25.66	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-	470.376	$C_{31}H_{50}O_3$	12.82

extracts APSE-2 (12.82%), 6-Octadecenoic acid (4) in fungal extracts APSE-1 (12.06%) and APLE-4 (11.91%), Bis(2-ethylhexyl) phthalate (5) in APLE-2 (11.7%), Cytochalasin H (6) in fungal extracts APLE-4 (10.15%) and APLE-3 (8.72%), Octadecanoic acid (7) in fungal extracts APSE-1 (10.09%), APLE-3 (8.37%), and APLE-4 (5.22%), Griseofulvin (8) in fungal extract APLE-1 (5.81%), Dechlorogriseofulvin (9) in fungal extract APLE-1 (5.55%) and Curvulin (10) in fungal extract APLE-2 (5.15%). Figure 16 illustrates the chemical structures of the principal bioactive compounds derived from plant and fungal sources.

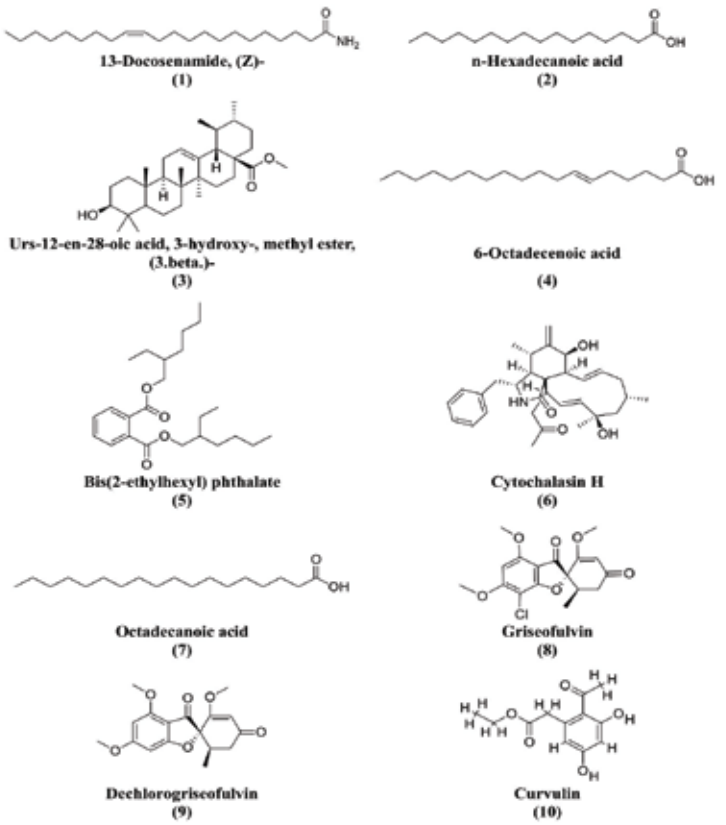

Fig. 16. Major bioactive compounds in the medicinal plant *Alternanthera philoxeroides* (Mart.) Griseb. (AP) and its associated endophytic fungi by GC-MS/MS analysis

GC-MS/MS analysis of the extracts identified diverse chemical constituents, including fatty acids (palmitic, stearic, and petroselaidic acids) and compounds with ester, ketone, amide, carboxyl, and amino functional groups. The compounds identified are believed to contribute significantly to the bioactivity exhibited by the extracts. Prior investigations have documented extensive pharmacological properties associated with the identified compounds. Within the context of this study, the most notable metabolite originating from fungal origin was identified as 13-Docosenamide, (Z)- (1). Compound 1, also called erucamide, has been documented to exhibit antioxidant, antimicrobial, cytotoxic, and anti-inflammatory properties (El-Gazzar *et al.* 2025; Khan *et al.* 2019, Rajalakshmi *et al.* 2024). Compound 1 is also identified by the fungal isolates APLE-1, APLE-2, APLE-3, APLE-4, and APSE-1 in minor quantities based on peak percentage area. n-Hexadecanoic acid (2), also known as palmitic acid, and contains a carboxyl group, as supported by the FT-IR study, and is produced by all crude extracts in significant quantities except APSE-2, which produces it in minor quantities and is documented to possess antibacterial, antifungal, antioxidant, and anti-inflammatory properties (Purushothaman *et al.* 2025; Behiry *et al.* 2024; Ganesan *et al.* 2024) in previous studies. Compound 3, Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-, an ester, confirmed by GC-MS/MS and FTIR is also documented previously and shown to have antibacterial potential (Abdelaziz *et al.* 2025, Uthirasamy *et al.* 2021). 6-Octadecenoic acid (4), also known as petroselaidic acid, which is found in almost all crude extracts based on minimum match factor, except APLE-1, and is reported to show COVID-19 inhibitor and antibacterial properties (Arundina *et al.* 2024; Ohtera *et al.* 2013). The fungal extract APLE-2 (10.77%) contains Bis (2-ethylhexyl) phthalate (5) in significant quantities, while APLE-1, APLE-3, APSE-1 contain in minor amount and is recorded to exhibit antibacterial and larvicidal Potential (Javed *et al.* 2022). Cytochalasin H (6) is found in the fungal extracts APLE-3 and APLE-4 that has also been identified from the endophytic fungus *diaporthe* sp., showing antibacterial and cytotoxic potential (Swandiny *et al.* 2025). All crude extracts but APSE-2 contain the octadecanoic acid (7), also known as stearic acid, has been found to display significant antibacterial activity from endophytic fungus from a previous report (Manganyi *et al.* 2019). Griseofulvin (8) and Dechlorogriseofulvin (9), antifungal, were found in significant amount in APLE-1. Compound 8 was present at low quantities in APLE-4 and APSE-1, which was derived from the endophytic fungus *Xylaria* sp. with notable antifungal effects (Park *et al.* 2005) and compound 9 was minor in APSE-1, which had antifungal potential (Zhao *et al.* 2012). A minor compound curvulin (5) has only been identified in fungal extrat APLE-2 (5.15%), which has been documented from two endophytic fungi exhibiting antileishmanial activity (Almeida *et al.* 2018). The strong antibacterial and antioxidant nature of *Curvularia* sp. (APLE-2 and APSE-2) might be due to the presence of compounds 1, 2, and 5; on the other hand strong antibacterial activity of *Diaporthe* sp. (APLE-4) might be related to the compounds 2, 6, and 7. *Xyliria* sp. (APLE-1) and *Colletotrichum* sp. (APLE-4) revealed modest antibacterial activity, which may be attributed to the presence of bioactive constituents 2, 6, and 7. The moderate antioxidant property of *Curvularia* sp. (APSE-1) might be due to the existence of compound 2.

However, further chemical investigations are essential to comprehensively characterize the metabolite profiles of these extracts. This study focuses on the isolation, identification, and evaluation of potential fungal endophytes associated with the medicinal plant *Alternanthera philoxeroides* (Mart.) Griseb., collected from a locally thriving population in Bangladesh. The findings demonstrate that fungal endophytes derived from this plant constitute a valuable source of bioactive compounds and hold promise for fulfilling the growing need for effective therapeutic agents.

Biological activities of fungal crude extracts

Antimicrobial assay

The disc diffusion methodology was employed for the assessment of antimicrobial activity. The fungal strains *Curvularia* sp. (APSE-1) and *Curvularia* sp. (APSE-2) demonstrated inactivity against all tested bacterial strains as well as other fungal strains, as delineated in Table III. The strain *Xylaria* sp. (APLE-1) exhibited mild antimicrobial activity against the bacterial strains *B. megaterium* (8.5 mm), *S. aureus* (9.5 mm), and *S. typhi* (9.5 mm), while no activity was recorded against the remaining bacterial and fungal strains. The fungal strain *Colletotrichum* sp. (APLE-3) displayed minimal activity against *B. megaterium* (6.5 mm), *P. aeruginosa* (7.5 mm), *E. coli* (7 mm), and *S. aureus* (12 mm). Conversely, the fungal strains *Curvularia* sp. (APLE-2) and the strain *Diaporthe* sp. (APLE-4) exhibited remarkable antimicrobial efficacy against all bacterial strains, with inhibition zones of *B. megaterium* (18.5 mm and 17.5 mm), *S. aureus* (26 mm and 16.5 mm), *P. aeruginosa* (29.5 mm and 25 mm), E. coli (17.5 mm and

15.5 mm), and *S. typhi* (17 mm and 16.5 mm) respectively, while no activity was observed against the fungal strains (Table III).

Antioxidant activity

The free radical scavenging activity of crude fungal extracts was evaluated using the DPPH assay. Among the isolated fungal strains, the crude extracts from *Curvularia* sp. (designated APSE-2 and APLE-2) exhibited particularly pronounced scavenging activity, exhibiting IC_{50} values of 42.43 μgml^{-1} and 47.83 μgml^{-1}, respectively, in comparison to the IC_{50} values of the positive controls, butylated hydroxyanisole (BHA) and ascorbic acid, which were recorded at 1.23 μgml^{-1} and 1.62 μgml^{-1}, respectively (Figure 7). Furthermore, the crude extracts from *Curvularia* sp. (APSE-1) and *Xylaria* sp. (APLE-1) demonstrated moderate scavenging activity, yielding IC_{50} values of 62.08 μgml^{-1} and 141.84 μgml^{-1}, respectively. Additionally, the crude extracts of the *Diaporthe* sp. (APLE-4) and *Colletotrichum* sp. (APLE-3) exhibited minimal scavenging activity, with IC_{50} values of 234.67 μgml^{-1} and 317.83 μ gml^{-1}, respectively. These findings indicate that *Curvularia* sp. recognized as (APLE-2) and (APSE-2) possesses a pronounced antioxidant activity, which may warrant its consideration as a source of metabolites with antioxidant properties potentially effective against various diseases induced by free radicals. Antioxidants play a critical role in inhibiting or postponing the oxidation of susceptible substrates in chain reactions, which is essential for maintaining optimal health (Mahmud *et al.* 2020).

The current investigation constitutes the first biological assessment of the crude extracts derived from six endophytic fungal species associated with *Alternanthera philoxeroides* in Bangladesh. Among the evaluated extracts, the isolate APLE-2, which has been categorized as *Curvularia* sp. at the genus level based on morphological analysis, demonstrated the highest potential among all tested extracts, exhibiting significant antioxidant efficacy and demonstrating an inhibitory effect on the proliferation of the five bacterial strains characterized as pathogenic. Numerous studies have documented the antibacterial and antioxidant properties exhibited by various endophytic fungi linked to distinct medicinal flora. *Curvularia species* are known to encompass several heterogeneous groups of secondary metabolites, including alkaloids, terpenes, polyketides, and quinones, which are associated with various biological activities such as anti-cancer, anti-inflammatory, antimicrobial, antioxidant, and phytotoxic effects (Mehta *et al.* 2022). For instance, the isolated *Curvularia* sp. from the medicinal plant *Rauwolfia macrophylla,* displayed antibacterial properties against all tested bacterial strains, including *Pseudomonas agarici*, *Escherichia coli*, and *Staphylococcus warneri,* and exhibited antioxidant activity as determined by DPPH and ABTS+ scavenging methodologies (Kaaniche *et al.* 2018) which supports our findings. The ethyl acetate extract of *Curvularia tsudae*, isolated from the perennial herb *Cynodon dactylon* (L.) Pers, exhibited moderate antimicrobial activity against Enterococcus faecalis, *Escherichia coli*, *Pseudomonas fluorescens,* and *Staphylococcus aureus.* Additionally, cyclic voltammetry analysis of the extract indicated significant antioxidant potential (Nischitha *et al.* 2020). The crude extract of secondary metabolites from the endophytic fungus *Curvularia* sp. G6-32, which features (-)-asperpentyn as a predominant metabolite, exhibits significant antioxidant activity (Polli *et al.* 2021). The extract from *Curvularia lunata,* identified from the aromatic grass *Cymbopogon caesius,* demonstrated antimicrobial properties against *Staphylococcus aureus, Salmonella typhi,* and *Escherichia coli,* exhibiting particularly high activity against *Staphylococcus aureus* (Avinash *et al.* 2015). *Colletotrichum gloeosporioides,* isolated from the medicinal plant *Cinnamomum malabatrum*, displayed antimicrobial activity against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis, Escherichia coli, Salmonella typhimurium,* and *Shigella boydii* (Packiaraj, 2016). Another study reported a novel endophytic fungus, *Colletotrichum gloeosporioides*, isolated from the medicinal plant *Vitex negundo* L., with extracts of *C. gloeosporioides showed* effective antimicrobial activity against bacterial strains such as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa* (Arivudainambi *et al.* 2011). An endophyte; *Colletotrichum* sp., reported from the medicinal plant of *Artemisia annua exhibited* activity against *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas* sp. (Lu *et al.* 2000). The EtOAc extract of the endophytic Fungus *Xylaria* sp. derived from *Andrographis paniculata* demonstrated antibacterial activity in a previous report (Suryelita *et al.* 2021). A study reported that Some compounds isolated from endophytic fungus *Diaporthe* sp. were active against diverse Gram-negative and Gram-positive bacteria (Sousa *et al.* 2016). The endophytic fungus *Diaporthe caatingaensis*, isolated from the medicinal plant *Buchanania axillaris,* exhibited antibacterial activity against all tested bacterial

strains at low concentrations of 12.5–25 µg/ml in a study conducted in India (Dhakshinamoorthy *et al.* 2021). Five fungal endophytes were successfully isolated from the medicinal plant *Otoba gracilipes* (Myristicaceae) and were taxonomically assigned to the genera *Xylaria* and *Diaporthe,* and subsequently subjected to screening to elucidate the promising potential of these microorganisms for the biosynthesis of bioactive secondary metabolites exhibiting extensive antibacterial properties (Charria-Girón *et al.* 2021). Our research suggests that the results obtained substantiate the capacity of *Alternanthera philoxeroides* to offer innovative strategies concerning the worldwide rise of antibiotic resistance and the prospective synthesis of novel compounds distinct from the antibiotic classes currently in use.

Conclusion

This manuscript represents the inaugural report concerning the isolation, identification, and initial bioactivity assessment of fungal endophytes pertinent to the medicinal species *Alternanthera philoxeroides* (Mart.) Griseb within the geographical context of Bangladesh. The findings elucidate that the amphibious weed *Alternanthera philoxeroides* (Mart.) Griseb. is a reservoir for numerous endophytic fungi with the potential to synthesize both antimicrobial and antioxidant compounds. GC-MS/MS-based chemical analysis elucidated the presence of a total of 47 distinct compounds within the crude extracts derived from fungal isolates and their associated hosts. The compounds identified, especially the predominant ones, have been previously reported to possess diverse pharmacological properties, which could explain the bioactivities observed in this study. Future studies could focus on the isolation and purification of bioactive compounds from these endophytic fungi, potentially providing a significant resource for the discovery and development of new pharmacological agents.; moreover, the biosynthesis of such compounds by fungi may facilitate the biotechnological mass production of viable alternative sources for antioxidants, antimicrobials, and various therapeutic agents.

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