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Research Article

Antibacterial and Secondary Metabolite Detection of Endophytic Fungi Isolated from the Roots of *Dendrocnide meyeniana* (Walp.) Chews

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ABSTRACT

Fungal endophytes have garnered considerable attention as promising reservoirs of novel bioactive compounds with antimicrobial properties. This study focuses on the isolation, morphological characterization, phytochemical analysis, and antimicrobial screening of fungal endophytes isolated from the roots of *Dendrocnide meyeniana*. There were 11 fungal *Dendrocnide* root endophytes (DRE) isolated wherein three were successfully purified and screened against *Escherichia coli* BIOTECH 1634 and *Candida albicans* ATCC 10231. Morphological examination of the isolates was identified as *Fusarium* sp. (DRE IS03), *Geotrichum* sp. (DRE IS05), and morphospecies *Mycelia sterilia* sp. (DRE IS07). By using the agar plug diffusion method, the zone of inhibition (ZOI) of the isolates DRE IS03 (26mm), DRE IS05 (26mm) and DRE IS07 (24mm) exhibited significant antibacterial activity against *E. coli* but no inhibitory effect in *C. albicans*. Furthermore, biochemical screening of DRE isolates unveiled the presence of diverse bioactive compounds, including saponins, steroids, and terpenoids, known for their antimicrobial properties. This study confirms the successful isolation of fungal endophytes from *D. meyeniana* roots, highlights the presence of bioactive fungal metabolites, and demonstrates antibacterial activity.

Keywords: Agar-plug diffusion assay, Ethnopharmacology, Manobo, Mt. Nebo, Talaandig

1. INTRODUCTION

Microbial endophytes live in and appear to be symbiotic with plants in a natural ecosystem (Rodriquez et al., 2009). Endophytes are present in various parts of the plants and contribute to growth, nutrition, and defense against plant pathogens (Yadav et al., 2014; Andreozzi et al., 2019; Shen et al., 2019). Fungal endophytes produce valuable compounds with biotechnological and industrial applications, such as antivirals, antioxidants, antibiotics, and insecticides (Gouda et al., 2016; Yadav, 2014). In the Philippines, several studies have demonstrated the diverse roles and potential applications of fungal endophytes, ranging from their biodiversity in unique plants and mangrove ecosystems to their pharmaceutical potential (Apurillo et al., 2019; Bibi et al., 2020; Petrini et al., 1992). Magday Jr. et al., (2023), explored the diversity of fungal endophytes associated with the Philippine endemic ginger, *Vanoverberghia sepulchrei* Merr., contributing to the understanding of fungal biodiversity in unique plant species and Jacob et al. (2023) compared the diversity and functional traits of fungal endophytes in response to elevated mineral content in a mangrove ecosystem, providing insights into how environmental factors shape endophyte communities. In a related study, Rondilla et al. (2022) identified *Annulohypoxylon stygium*, a fungal endophyte associated with *Pandanus simplex*, which exhibited α-glucosidase inhibitory activity, demonstrating its potential for pharmaceutical applications. De Mesa et al. (2020) investigated the antagonistic activities of needle-leaf fungal endophytes against *Fusarium* spp., underscoring their potential as biocontrol agent. Furthermore, dela Cruz et al. (2020) examined the biomining potential of fungal endophytes from tropical plants and seaweeds for drug discovery, emphasizing the vast untapped potential of fungal biodiversity in biomedicine. Thus, synthesis of ingredients from natural compounds is significant. In the study of Bungihan et al., (2013) several fungal endophytes isolated from the mature leaves of *Pandanus amaryllifolius* showed a wide array of secondary metabolites belonged to the class of phenolics, coumarins, sterols and terpenoids. Phytochemical analysis of *Dendrocnide sinuate* revealed the presence of carbohydrate, tannin, flavonoid, phenol, terpenoids, cardiac glycoside, and alkaloid (Aquino et al., 2018; Borah, et al., 2023). In addition, *Dendrocnide meyeniana* was also investigated using chloroform+methanol as solvent system and the result revealed the presence of saponins, phenols, tannins, flavonoids, anthrones, anthraquinones, terpenes and steroids which are considered as active medicinal phytochemical constituents (Aquino et al., 2018; Torno et al., 2024). Their findings suggests that there is a need for further studies on metabolites from other plants as potential sources of medically important metabolites to understand the relationship between the fungal endophytes and its host plant that harbour potential medicinal compounds (Guerrero et al., 2019; Selim et al., 2012; Strobel et al., 2003). Moreover, unlike other natural sources, fungal endophytes provide a renewable and sustainable way to produce valuable metabolites without relying on overharvesting plants or other organisms from the wild (Strobel and Daisy 2003; Yang et al., 2017). Moreover, fungal endophytes come from unique and unexplored environments that may increase the likelihood of discovering novel compounds and can be optimized for production of desired secondary metabolites (Selim et al., 2012; Yang et al., 2017). Since there is insufficient knowledge regarding the fungal endophytes of *D. meyeniana* particularly in relation to antimicrobial assessment and phytochemical determination, this study aims to determine the bioactive secondary metabolites produce by its endophytic fungi. *Dendrocnide* species within the Urticaceae family is a type of stinging tree found in Northern Australia and Southeast Asia (Gunardi, et al., 2023; Schmitt et al., 2013). *Dendrocnide* possess stinging trichomes on its juvenile leaves, which contain a toxin that can cause prolonged discomfort and a stinging sensation upon contact with human skin (Fu et al., 2002; Schmitt et al., 2013).

2. METHODOLOGY

Permits and Study Site: Necessary permits were secured such as DENR Gratuitous Permit (R10-2024-09) through PAMB Presentation, Certification and Prior Informed Consent (PIC) from the barangay officials of Mt. Nebo, Valencia City, Bukidnon, and the tribal communities before the interview and collection of plant sample. Mt. Nebo is situated in the highland region of Bukidnon, an agricultural land and with some patches of secondary forests and grasslands, characterized by a relatively cool climate due to its elevation 896.5 meters above sea level (7.9728N, 124.9866 E), with average temperature range between 20°C to 25°C, and annual precipitation averaging around 2,000 to 2,500 mm (Philatlas, 2015).

Collection and Identification of host plant: Ten mature and healthy plants of *D. meyeniana* growing around Mt. Nebo were collected between November 2023 to February 2024. Collected plants were placed in brown paper bags and transported to the laboratory for isolation of endophytes. Voucher specimens of *D. meyeniana* were deposited in the Central Mindanao University Herbarium (CMU) and folk names were compared to the Dictionary of Philippine Plant Names by Madulid (2001). The herbarium specimen was identified and certified by Prof. Lowell G. Aribal, a Plant Taxonomists of the College of Forestry and Environmental Science, Central Mindanao University, Philippines.

Isolation and Identification of Fungal Endophytes: The root samples of *D. meyeniana* were cut into 5 cm length segments and subjected to surface sterilization using a modified procedure of Sahu et al. (2022). The cut segments underwent sequential surface sterilization, which involved immersions in sterilized distilled water for 1 minute, 70% ethanol for 2 minutes, sterilized distilled water for 1 minute, 2.5% sodium hypochlorite for 3 minutes, sterilized distilled water for 1 minute, and 70% ethanol for 30 seconds. The root segments were then rinsed thrice with sterilized distilled water for 1 minute each to remove any remaining chemicals. Tissue plating was done to determine the effectiveness of the surface-sterilization wherein the surface-sterilized roots were cut into approximately 2 mm^2 length and the explants (10 explants per plate) were placed on fresh Potato Dextrose Agar (PDA) supplemented with 50 mg/L tetracycline (Sigma) and 10 mg/L streptomycin (Sigma). Hyphal tips from the fungal colonies were transferred to freshly prepared plates of PDA augmented with 10 mg/ml of streptomycin to inhibit bacterial growth. After incubating for seven days at 30°C, the fungal edges were trimmed and re-isolated on PDA to obtain pure cultures. The said process was repeated three times until colonies with the same appearances from previous isolates emerged. By comparing their morphocultural characters with those of fungi in published literature the fungal isolates on *Dendrocnide* roots endophytes (DRE) were identified (Barnett and Hunter 1998).

Metabolite production. The extraction of fungal metabolites from *D. meyeniana* roots were performed based on the protocol of Thirawatthana et al. (2013) with slight modifications. Three agar-plot samples from the edges of growing cultures were then cut out for each selected endophytic fungus. Selected fungal morphospecies of DRE were grown on Potato Dextrose Broth (PDB, Hi-media)) and cultured for seven days at 30°C and 280 rpm. Selection for mass propagation was based on their morphocultural characteristics, i.e. fungal isolates exhibiting different colony morphologies. After incubation, the culture was harvested using a filtration vacuum to separate the supernatant and mycelia. The mycelia and spores were dislodged using the inoculating loop, and this fungal suspension served as the inoculum. The fungal inoculum was then transferred onto 250 ml PDB and the cultures were incubated at room temperature for 2 weeks without shaking. Following incubation, the mycelial biomass was harvested by filtration and soaked overnight with 1:1 v/v ethyl acetate. To obtained the crude extract for antimicrobial test, the ethyl acetate extracts from the soaked mycelia were then concentrated in rotary evaporator until a syrupy formation was observed. Twenty milligrams of syrupy extracts were diluted with 1 mL acetone and methanol (1:1) to a final concentration of 20mg/ml to serve as the working concentration for antimicrobial assay.

Microbial strains and medium: The *Escherichia coli* BIOTECH 1634 and *Candida albicans* ATCC 10231 were obtained from the Philippine National Collection of Microorganism, University of the Philippines Los Baños (PNCM-UPLB). Nutrient-rich (NR) medium was used in inoculation, maintenance, and storage of the bacterial strains and contained 1% yeast extract, 1.5 nutrient broth, and 0.2% ammonium sulphate while the fungal isolate was cultivated on PDA.

Morphological Examination of Fungal Endophytes. Microscopic characteristics were examined by employing the slide culture method. A 5-mm square of potato dextrose agar medium was transferred to the centre of the glass slide. The spores or mycelia of the endophytic fungi were placed on the four sides of the agar square, and a cover glass was placed on the petri dish. The petri dish was then incubated at 30°C for 48 hours. After the incubation, the cover glass was removed and a lactophenol cotton blue was added. The morphological characterization of the isolated endophytes was observed which includes the following characteristics: spore, colonies found on the upper surface and reverse surface, hyphae color, type of hyphae, and hyphae shape (Barnett and Hunter, 2000).

Screening for Antimicrobial Activity: The antimicrobial activity of fungal endophytes isolated from the roots of *D. meyeniana* was screened using the agar plug diffusion method, following the procedure by Balouiri et al. (2016) with slight modifications. A 12-hour old culture of *E. coli* BIOTECH 1634 and 24-hour *C. albicans* ATCC 10231 was suspended in distilled water and the cell density was adjusted to 0.5 McFarland standard. Using cotton swab dipped into the cell suspension, the test organisms were spread plated onto the Mueller Hinton Agar (MHA) and Mueller Hinton Agar with 2% glucose and methylene blue surfaces. An agar plug culture of the selected strains were prepared on the surface of PDA plates. After incubation for seven days at 30°C, a sterile cork borer was used to aseptically cut an agar plug which was then placed on different petri plates with Mueller Hinton Agar (MHA) and Mueller Hinton Agar with 2% glucose and methylene blue as suggested by Berkow et al., 2020) based on the recommendation of the European committee on antimicrobial susceptibility testing (EUCAST). This method facilitated the rapid selection of endophytic fungal isolates against gram-negative bacterium (*E. coli* BIOTECH 1634) and unicellular fungus (*C. albicans* ATCC 10231) based on the appearance of inhibition zones around the agar plug. All culture plates (in triplicates) were incubated at 30 °C for 24-48 hours, and the zone of inhibition (ZOI) were

measured using a Vernier caliper (3 readings per plate). The negative control (solvent) was a basis for the computation for the zone of inhibition by deduction of the ZOI measured around the plug of soaked with fungal extracts. The positive control used was ciprofloxacin (Sigma) for *E. coli* and vericonazole (Sigma) for *C. candida* at a concentration of 1mg/mL. The antibacterial activities were evaluated based from Quinto and Santos (2005) category as: (1) very active (>41mm ZOI), (2) active (28-41mm ZOI), (3) partially active (21-27mm ZOI), and (4) inactive (<21 mm ZOI).

Preliminary Phytochemical Screening. The fungal endophyte extracts were subjected to standard phytochemical tests to evaluate their chemical composition for different active constituents; for these extracts (3–5 mg/mL), they were separately suspended in 1 mL of absolute ethanol or distilled water using clean test tubes as described Bhardwaj (2015). For alkaloids, fungal extract was dissolved in a 2N HCL solutions, treated with a few drops of Mayer's reagent, formation of a creamish precipitate indicate the presence of alkaloids. To detect the presence of flavonoids, fungal extract was added with a few drops of 20% NaOH, resulting in a yellow color, which turned colorless upon addition of acid. For phenols, fungal extract was dissolved in a 5 ml of distilled water, followed by the addition of neutral 5% ferric chloride solution, resulting in a dark green color, indicative of phenols. The saponins were identified by frothing test, where vigorously shaking of extracts with distilled water and standing for 10 minutes led to the formation of stable emulsion, indicating their presence. Steroids were detected using the Libermann-Burchard reaction, fungal extract was added chloroform solution, treated with acetic anhydride and concentrated H_2SO_4, the formation of a blue green ring indicates the presence of steroids. Finally, for terpenoids, the fungal extract was mixed chloroform, followed by concentrated H_2SO_4, formation of a reddish-brown precipitate at the interface indicates the presence of terpenoids.

3. RESULTS AND DISCUSSION

Based from ethnopharmacological study of Dapar et al., (2023; unpublished data) on the traditional practices of the Manobos and Talaandigs community from selected areas of Mt. Kalatungan and Mt. Kitanglad, Bukidnon, Philippines, one of the highest-ranked medicinal plants based on its use report (UR) and use value (UV) is *D. meyeniana* (Walp.) Chew, locally known as "sagay sagumbilin," which is commonly employed for the treatment of urinary tract infections (UTI), diarrhea, fatigue and hypertension. However, to best of our knowledge there are only few studies on the economic importance of this plant. *Dendrocnide* species is notorious for causing skin irritation, a stinging sensation, and prolonged discomfort when leaves encounter human skin due to the presence of stinging trichomes on their juvenile leaves that contain toxins (Fu et al., 2002; Schmitt et al., 2013). Its phytochemical analysis revealed the presence of carbohydrate, tannin, flavonoid, phenol, terpenoids, cardiac glycoside, and alkaloid (Torno et al., 2024). *Dendrocnide* is the only reported genus that produced pain-causing peptides, namely moroidin and gympietides as healing capacity for antidiabetic, antiulcer, antibacterial, cardiovascular-related activities, brain disorder, allergic rhinitis-related activities, and anticancer activities (Gunardi et al., 2023). *Dendrocnide sinuate* (Blume) showed the anti-inflammatory activity by stabilizing the human red blood cell membrane at 150ug/ml (Borah et al., 2023). *D. meyeniana* root extracts have anti-angiogenic properties and these findings will help to understand the efficacy of the traditional medicine used by the local people (Torno et al., 2024). Unfortunately, there is also limited knowledge regarding the fungal endophytes associated with *D. meyeniana*, particularly in assessment of their antimicrobial properties and its phytochemical composition. In this study, seven pure cultures of endophytic fungi isolated root segments of *D. meyeniana* based from its colony morphology, color, and size (Table 1 and Figure 1).

Table 1. *Macroscopic characteristics of endophytic fungal isolates from D. meyeniana*

Endophytic Fungal Isolate	Colony Formation	Colony Elevation	Colony Texture	Colony Margin	Pigmentation	
					Obverse	Reverse
DRE IS01	Circular	Raised/Umbonate	Glabrous (smooth)	Entire	Cream-white	White
DRE IS02	Filamentous	Raised/Umbonate	Cottony	Entire	Cream-white	White
DRE IS03	Filamentous	Raised/Umbonate	Cottony	Filliform	White	White-orange
DRE IS04	Filamentous	Raised/Umbonate	Cottony	Filliform	White	White
DRE IS05	Circular	Flat	Glabrous (smooth)	Entire	Cream-white	White
DRE IS06	Filamentous	Raised/Umbonate	Cottony	Entire	Cream-white	White

DRE IS07	Filamentous	Raised/Umbonate	Cottony	Filliform	White	Slightly pale

Based from the macroscopic observation, these isolates were divided into three main groups according to their colony color and were further selected for microscopic observation for identification. Additionally, all isolates studied in this project were deposited in the fungal culture collection of the Natural Science Research Center, Central Mindanao University, Philippines.

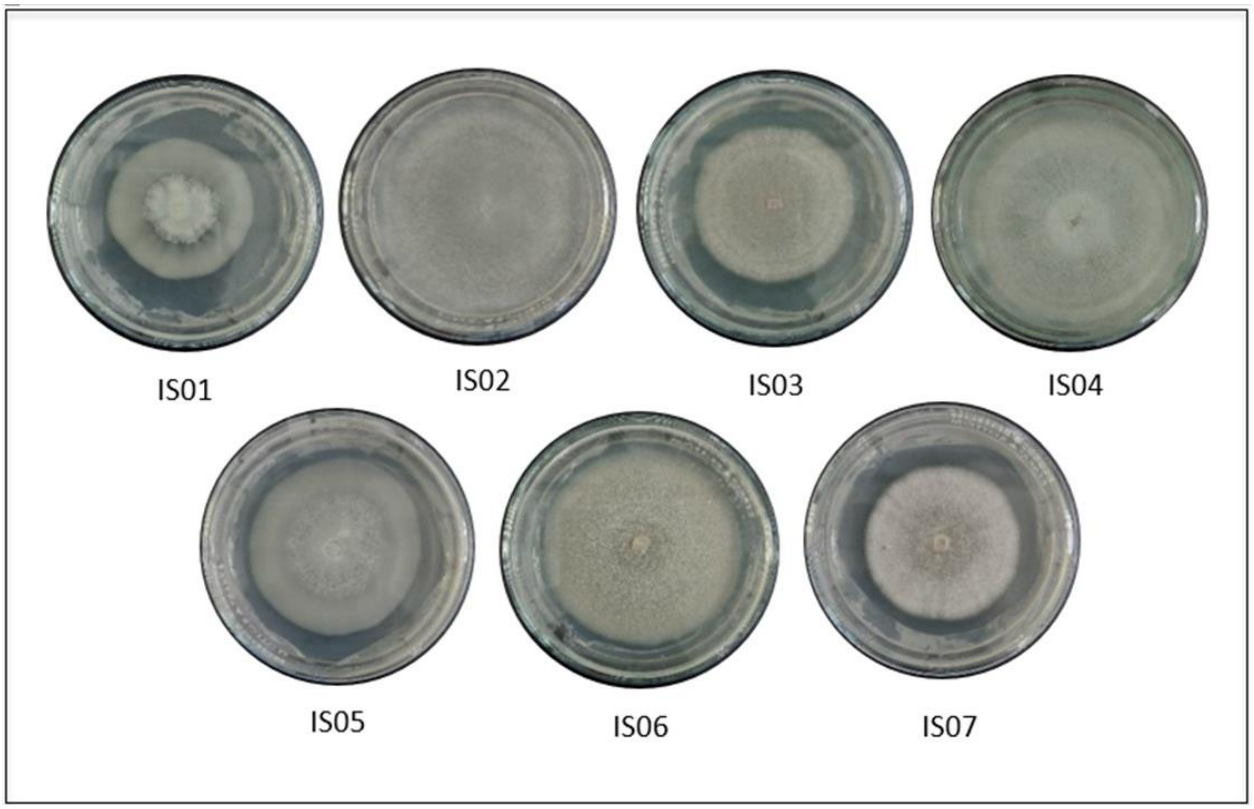

Figure 1. *Endophytic fungi isolated from the roots of D. meyeniana inoculated on PDA plates at 30°C for seven days.*

The colony of DRE IS01, DRE IS02, DRE IS04, and DRE IS05 isolates were creamy white on PDA medium while the color of the back of the plate is white and exhibit rapid development, appearing as filamentous and displaying a texture ranging from velvety to cottony. The colony of DRE IS03 and DRE IS06 also exhibit fast growth and have a circular shape with a glabrous (creamy) texture, and the conidia/spores are visibly present on stained septate mycelia, and fruiting bodies are produced. Moreover, their arthroconidia are observed and are often abundant, occurring as individual structures. Meanwhile, the DRE IS07 conidia/spores are not visible on stained septate mycelia and no fruiting bodies are observed. Based on these colony and culture properties, three isolates were selected for antimicrobial and phytochemical analysis. The DRE fungal isolates were identified on the basis of their cultural and microscopic characteristics (shape and size of spores, hyphae) by following standard monographs and taxonomic manuals. DRE IS03 shares identical characteristics with *Fusarium* sp. based on observed characteristics (Nucci et al., 2021; Nozawa et al., 2023), DRE IS05 resemble those of *Geotrichum* sp. (Tshisevhe et al., 2021), and DRE IS07 are similar with morphospecies *Mycelia sterilia* (Guo et al., 2000). All three (3) selected endophytic fungal isolates were morphologically characterized and confirmed using an identification guidebook by Barnett and Hunter (2000) (Table 2 and Figure 2). This guide includes examining of various characteristics such as hyphae, hyphal form, conidia shape, spore type, upper and reverse surface of colonies, type of concentric growth, hyphal color, type of hyphae and hyphal shape.

Table 2. *Microscopic characteristics of endophytic fungal isolates DRE IS03, DRE IS05 and DRE IS07*

Endophytic Fungal Isolate of *D. meyeniana*	Hyphae (septum)	Hyphae (pigmentation)	Spore type	Prospect Genus Identification (Barnett and Hunter, 2000)
IS03	septate	raised/umbonate	microconidia	*Fusarium* sp.
IS05	septate	Flat	arthroconidia	*Geotrichum* sp.
IS07	-	-	-	Morphospecies *mycelia* sp.

However, molecular identification based on sequencing the internal transcribed spacer (ITS) and large subunit (LSU) regions must be conducted to complete the morphological identities. There are several advantages for the molecular identification including conservation within species (variable among different species), presence in multiple copies in the genome allowing for more sensitive detection and quantification of fungal DNA, and relatively easy to amplify by PCR using universal primers, which leads to suitable amplicons for sequencing. In concurrence with our findings, various *Fusarium* spp., *Geotrichum* spp., and *Mycelia* morphospecies, have been isolated and identified as an endophytic fungus of *Pandanus amaryllifolius* (*Pandanaceae*) including several species within the genera of *Fusarium* and *Geotrichum*, and other mycelial morphospecies (Dela Cruz et al., 2006) and some selected medicinal plants in the Philippines (Quimio, 2001). Additionally, the study of Lubo et al., (2018) have isolated endophytic fungi from different plant parts of *Tithonia diversifolia* in the Philippines including various mycelial fungi. Recently, a study on the diversity of fungal endophytes, including *Fusarium* and other filamentous fungi were isolated from grasses in the Mt. Makiling Forest reserve (Monggo et al., 2022).

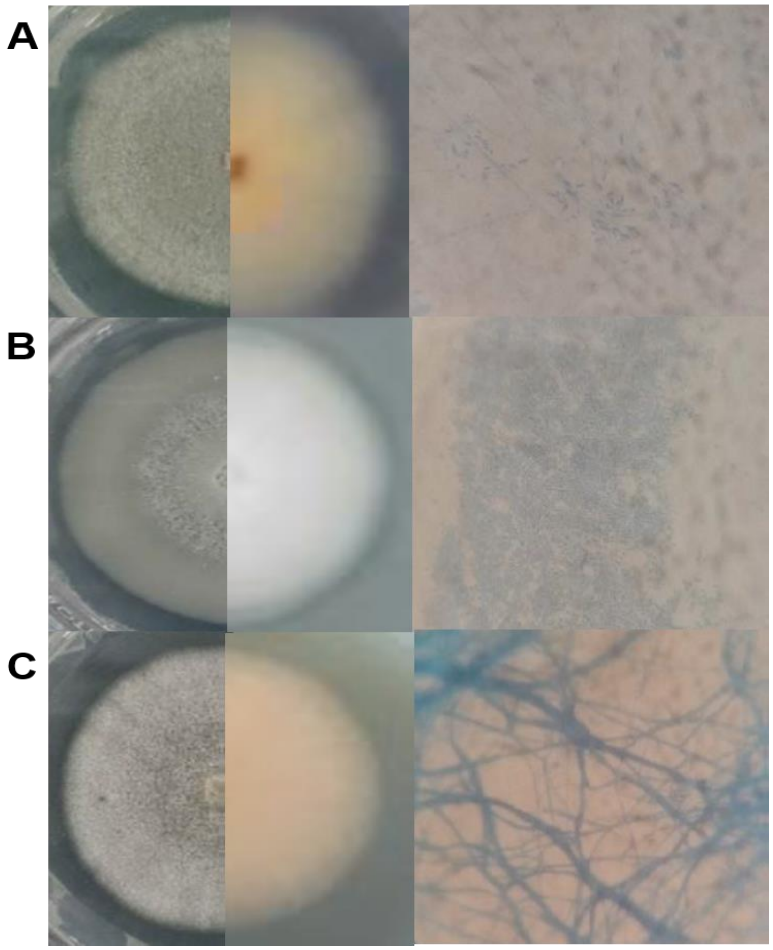

Figure 2. *Representative microscopic view of DRE fungi isolates grown in PDA plates at 30°C, 7days (a. DRE IS03, b. DRE IS05, and c. DRE IS07)*

Accordingly, endophytic fungi are sources of novel secondary metabolites with different biosynthesis pathways were most of these compounds can be biologically active against a wide array of microorganisms (Schulz et al., 2005;

Bhalodia & Shukla, 2011). In this study, of the seven endophytic fungi, there were six that exhibited antibacterial activity against *Escherichia coli* BIOTECH 1634 while there is none for *C. albicans* (Figure 2). The antibacterial potential of the isolated fungal endophytes revealed a substantial inhibitory activity against *E. coli*. Based from the criteria set by Quito and Santos (2005), the DRE isolates have ZOI ranges from 22-26 mm implying that they can be partially active against *E. coli*. Similarly, Bungihan et al., (2013) fungal metabolites from *P. amaryllifolius* endophytes, particularly the crude culture extracts of *Colletotrichum* spp. (PLE 13, PLE 14 and PLE 45) and *Chaetomium* sp. (PLE 56) showed antibacterial activities against *S. aureus, E. coli* and *G. terrae*. Apparently, in this study, no inhibitory activity was observed against *C. albicans* among the isolates compared to the study on the antifungal activity of endophytic *Fusarium oxysporum* from *Cola acuminata* against *Candida albicans* isolates from HIV-infected patients showing significant that antifungal activity (Fogue et al., 2022). Further investigation must be done to understand the underlying mechanism on the factors involving the antimicrobial activity of DRE isolates. Maybe metabolites produced by these may act by interfering with bacterial DNA replication, enzyme function, or membrane integrity, which are distinct from the targets required to disrupt fungal cells like *Candida albicans*. This selectivity limits the cross-effectiveness of these isolates' metabolites.

Figure 3. *Agar plug assay on Mueller Hinton agar of seven endophytic fungal isolates DRE IS02 (24mm), DRE IS03 (26mm), DRE IS04 (24mm), DRE IS05 (26mm), DRE IS06 (22mm), and DRE IS07 (24mm) against E. coli BIOTECH 1634*. Zones of inhibition were observed after 24 hours of incubation at 37°C.

A qualitative biochemical study was done to determine the presence of secondary metabolites of the DRE fungal isolates that are morphologically characterized as *Fusarium* sp. (DRE IS03), *Geotrichum* sp. (DRE IS05), and *mycelia sterilia* (DRE IS07). The study revealed that the fungal isolates have saponins, steroids, and terpenoids (Table 3) (Masyita et al., 2022; Senguttuvan & Paulsamy, 2014; Singh et al., 2021; Vollaro et al., 2020). However, alkaloids, flavonoids, and phenols were not detected during the qualitative phytochemical analysis in contrast to the study of Ahmed et al., (2023) on the natural products detected from endophytic fusarium. Among the endophytic fungal isolates, DRE IS05 exhibited a higher number of secondary metabolites, with a significant degree of precipitation, saponins and terpenoids were found in lesser amounts in the culture media. The presence of several secondary compounds which are naturally occurring in most plant extracts were identified to possess antimicrobial properties on microbial pathogens as well (Lu et al., 2012; Solis et al., 2016; Xingyuan et al., 2022).

Table 3. *Qualitative phytochemical analysis of endophytic fungal isolates of D. meyeniana*

Endophytic Fungal Isolate	Secondary metabolites					
	Alkaloids	Flavonoids	Phenols	Saponins	Steroids	Terpenoids
DRE IS03	-	-	-	-	+	++
DRE IS05	-	-	-	+	+++	+++
DRE IS07	-	-	-	+	-	+

Note. +++ indicates strong intensity, ++ indicates moderate intensity, + indicates mild intensity, - indicates negative.

The endophytic microorganisms inhabiting the plant tissues are expected to mimic some of the metabolites of its host. Several identified metabolite groups, such as saponins, steroids, and terpenoids, are widely recognized for their strong antibacterial and antifungal properties (Da Silva et al., 2016; Assaf et al., 2020). The mentioned findings offer a plausible explanation for the observed antibacterial activity exhibited by the three endophytic isolates. In addition, the qualitative phytochemical analysis of DRE fungi isolates revealed the presence various phytochemicals such as saponin, steroids, and terpenoids that could potentially exhibit antimicrobial activities (Dubale et al. 2023; Al-Daihan et al. 2013; Hussain et al. 2011). Further investigation must be done to observed other biological activity in this plant that may attribute to the presence of various chemical classes for the synergistic interactions among these bioactive compounds and the ones present in the isolated *D. meyeniana* fungal endophytes. The findings in this study could provide scientific validation for the traditional uses of *D. meyeniana* and hold promise for the future of antibacterial drug research.

4. CONCLUSION

The present study showed that the *D. meyeniana* present a wide array of endophytic fungi particularly associated in the roots as determined through morphological and molecular techniques. The fungal endophytes secrete different secondary metabolites that have antibacterial activity. Out of the 11 fungal endophytes isolated, three were successfully purified and demonstrated notable antibacterial activity against *E. coli* while no inhibitory effect was observed against *Candida albicans*. These findings suggest the need for further studies in understanding the mechanisms on the symbiotic relationship of the host plant and its endophytes in plant growth and nutrient uptake, antimicrobial activity, and how it induces resistance against biotic and abiotic environmental stresses.

Author Contributions: LGMR, MLGD, and MGM: conception and design of study. JBM, CRML, NEL, ABM, WMG, RAL and MLGD: acquisition of data, writing—provided deeply discussion and sorted out the references. LGMR, and MGM: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The authors confirm that the data supporting the findings of the study are available within the article. Raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Conflicts of Interest: The authors have no competing interests to declare that are relevant to the content of this article.

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