



Original article

Diversity of endophytic fungi from medicinal plant *Oxalis latifolia* and their antimicrobial potential against selected human pathogensJ.M. Hussein^{*}, H. Myovela, D.D. Tibuhwa

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ARTICLE INFO

Keywords:

Endophytic fungi
 Medicinal plant
Oxalis
 Fungal diversity
 Molecular phylogeny

ABSTRACT

Endophytic fungi that inhabit medicinal plants are microbial resources renowned for having compounds analogous to those produced by their host plants. This study aimed to describe the diversity of endophytic fungi found in *Oxalis latifolia* Kunth. To better understand the diversity of foliar endophytic fungi found in the leaves of the medicinal plant *Oxalis latifolia*, we isolated and characterized endophytic by using both morphological and molecular methods employing ITS markers. The antimicrobial activity of endophytic fungi against common human pathogens *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* was also investigated. A Total of 16 endophytic fungi were successfully isolated from leaves and classified into five orders of Pezizomycotina based on the phylogenetic analyses; *Xylariales* (56%), *Diaporthales* (19%) *Sordariales* (6%), *Glomerellales* (13%) and *Botryosphaeriales* (6%). The antimicrobial activity of crude extracts from fungal endophyte against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* revealed that three isolates; *N. aurantiaca*, *Phyllosticta capitalensis* *N. oryzae* were the most potent, while *Colletotrichum karstii* and *N. sphaerica* displayed no growth inhibition property against the tested organism. The diversity indices were calculated by using the Shannon-Wiener, Margalef, and Simpson indices. The diversity indices analysis revealed an abundance of species diversity, where the dominant species were *Nigrospora oryzae*, *N. sphaerica*, and *Colletotrichum karstii*. This study describes the diversity of endophytic fungi found in *O. latifolia* and emphasizes their potential as a source of novel bioactive compounds. More research on phytochemical composition and antimicrobial activity is ongoing to correlate the traditional uses and scientific findings.

1. Introduction

Endophytic fungi reside in all or fragments of their life cycle in healthy plant tissues with no harm (Azevedo et al., 2000). They are abundant and inhabit the host plants, so one plant may hold over 30 fungal species (Rodriguez et al., 2009). They protect the plant against pathogens and adverse conditions by producing Phytohormones and chemicals (Selim et al., 2011; Mwanga et al., 2019). Due to their involvement in the host plant protection, endophytic fungi influence the plant community's diversity and structure (Aguilar-Trigueros & Rillig, 2016). Therefore, the study of endophytes associated with the plant of interest is a crucial step for the exploitation of the plant's secondary metabolites.

Medicinal plants are well-known sources of beneficial bioactive compounds for treating many ailments. They have been used as a significant source of health care, especially in developing countries where most people cannot afford hospital costs (WHO, 1995, Unnikrishnan,

2010). Research on bioactive compounds derived from medicinal plants has led to the introduction of novel drugs with high medical potential (Debbab et al., 2012). Endophytic fungi in a medicinal plant produce similar bioactive compounds as the host plant, according to research. (Strobel et al., 1999; Zhao et al., 2019). These findings reduced plant overexploitation due to the biotechnological use of endophytes in bioactive compound production (Zou et al., 2000; Tejesvi et al. 2007). In addition, studies on endophytic fungi have contributed primary taxonomic data on the distribution, diversity, and novel species description (Zhou and Hyde, 2001; Rhoden et al., 2012). Exploring the diversity of endophytic fungi in medicinal plants is therefore critical to discovering new sources of bioactive compounds and protecting endangered medicinal plants.

Oxalis latifolia Kunth is well-known in Tanzania for its traditional uses and diverse biological activities. The leaves of *O. latifolia* are used to treat a variety of human ailments consumed as a medicinal plant (Tibuhwa, 2016; Krishnan & Muruges, 2019; Tropical Plant Database

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2024). Previous reports have uncovered traditional uses in different ethnic groups in Tanzania and provided scientific evidence of its antibacterial activity and safety for consumption (Mwanga et al., 2019). *Oxalis latifolia* has been reported to be used traditionally for the treatment of ulcers, wound healing, dysentery, quarten fever, and skin diseases (<https://www.socfindoconservation.org/>; Tropical Plants Database). Previous studies have also reported antioxidant, anticancer, and antimicrobial activities of the extract of *O. latifolia* (Subramanian, 2018; Krishnan & Murugesu, 2019). Despite their importance in curing different diseases, endophytes associated with *O. latifolia* are poorly explored. Therefore, in this study, we explored the biodiversity of the endophytic fungi related to *O. latifolia* and assessed their bioactive compound production potential for the selected human pathogens.

2. Material and methods

2.1. Plant materials and study site

Plant samples were obtained from Bungu village which is located in the Korogwe District of Tanga, Tanzania. A total of 45 plant leaves were collected from five different areas where they grow (three leaves per plant, three plants per location). Plant samples were authenticated by a specialist from the Botany Department of the UDSM. The samples were then carried to laboratories in plastic bags and stored at 5 °C until used.

2.2. Endophytic fungi isolation

The leaves of *O. latifolia* were a source of isolated Endophytic fungi following the methods explained by Kjer et al. (2010) and Myovela et al., (2024). The samples were surface sterilized by cleaning with tap water to remove debris, followed by dipping in 70 % ethanol for 1 min, then treated with 3.5 % NaOCl solution for 1 min washed with distilled water, and finally blotted on a sterilized paper towel. The final rinse water was used as a control, where it was plated. Incubated and examined for fungal growth to indicate the sterility of the sample surface. Aseptically 1–2 mm of the sterilized segmented plant were inoculated on potato dextrose (PDA) wrapped with Parafilm before incubated at 27 °C for 7 days. The Petri dishes were examined regularly to check the progress of endophytic fungal colonies (Fig. 1). The growth that originated from incubated leaf segments was subcultured into fresh PDA media, followed

by subculturing until the pure culture was obtained. The isolates were morphologically and molecularly analyzed. For molecular analyses, genomic isolates were subcultured into Malt extract broth (MEB) for three days before DNA extraction.

2.3. Morphological endophytic fungi identification

Morphological identification was made using both macroscopic and microscopic features. For macroscopic characteristics, endophytes were characterized based on colony appearance, mycelial growth pattern, margin, and pigmentation, while microscopic analysis involved mycelia preparation on slides and staining them with the lactophenol-cotton blue reagent. Features such as hyphal type, septate or aseptate features, spores type, sporangia, conidia, and arrangement of sporangio-phores and conidiophores were observed.

2.4. DNA extraction, amplification, and sequencing

The pure culture of endophytic fungi was inoculated into 50 conical flasks containing 30 ml MEB and placed on a shaker incubator at room temperature to grow for 3 to 7 days. Extraction of genomic DNA was according to CTAB protocol. The endophytes culture grown in PDB was transferred to a 2 ml Eppendorf tube using a sterile micropipette tip; it was then homogenized using a sterile paste and the lysis buffer (20 mM EDTA, 100 mM Tris HCl, 2 % CTAB). Sterile glass beads were added, and homogenized mixture was followed by shaking in a dismembrator then incubated in a water bath set at 65 °C for 60 min. Respective volume of chloroform: isoamyl (24:1 v/v) was added and mixed by inversion. The mixture was centrifuged at 13,000 rpm for 15 min, after which the aqueous layer was carefully taken to clean 1.5 ml Eppendorf tube. An estimate was made for the volume of the aqueous phase, and half that volume (approximately 350 µl) of 6 M Sodium Chloride was added and mixed well. Then 70 µl of 3 M CH₃CO₂K was added and mixed with 500 µl chilled isopropyl alcohol inverted gently to precipitate DNA and then stored at – 20 °C for 30 min. The sample was centrifuged at 13,000 rpm for 5 min, and the pellet with DNA was cleaned with 70 % ethanol centrifuged at 13,000 rpm for 5 min. The supernatant was discarded, while the pellet was kept at room temperature for evaporation. A volume of 50 µl nuclease-free water was added to elute the DNA. The DNA quality and concentration were checked by 1 % agarose gel and

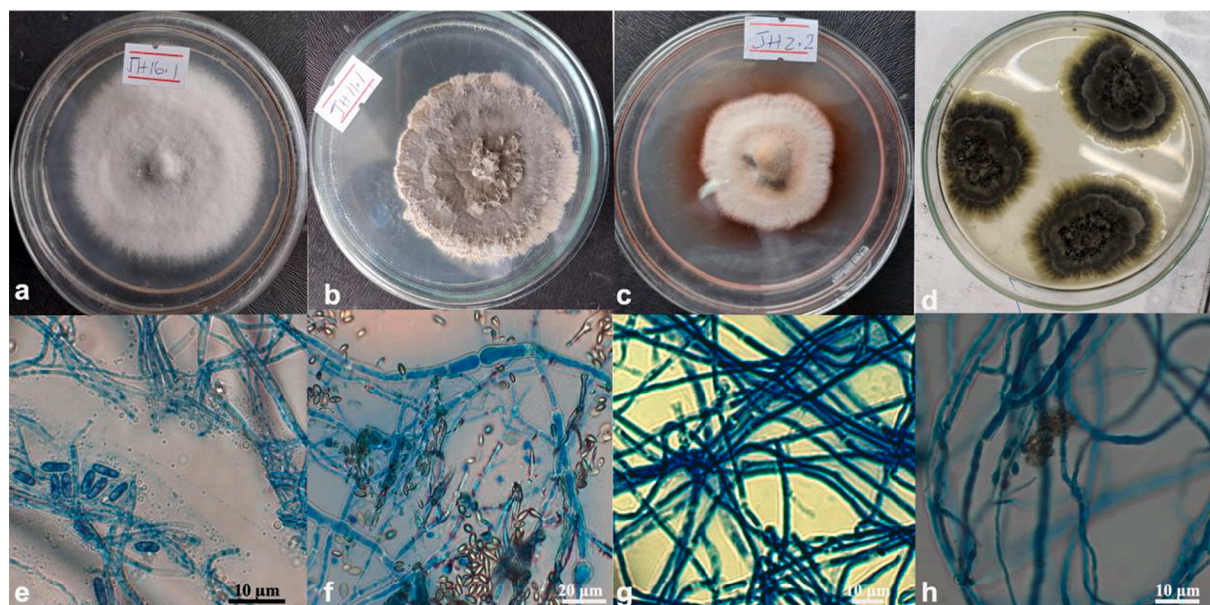


Fig. 1. Fungal endophytes isolated from the *Oxalis latifolia* leaves (a) *Colletotrichum karstii* (b) *Diaporthe eugeniae* (c) *Nigrospora aurantiaca* (d) *Phyllosticta capitalensis*. e-h showing microscopic features of *C. karstii*, *D. eugeniae*, *N. aurantiaca* and *P. capitalensis* respectively.

Nanodrop spectrophotometer (NanoDrop One Thermo Fisher Scientific), respectively. Polymerase chain Reaction (PCR) amplification was done by the ITS rDNA region using ITS4 (TCC TCC GCT TAT TGA TAT GC) and ITS 1 (TCC GTA GGT GAA CCT GCG G) primers as explained by White et al. (1990). Amplification was achieved in 25 µl reactions using a taq master mix, DNA template concentration of 50–100 ng/µl was used. The PCR was done using a thermocycler (Applied Biosystems model No 9902) following Hussein et al. (2018): Briefly, initial denaturation at 95 °C for 30 sec, 35 cycles of denaturation for 20 s at 94 °C, annealing for 30 s at 56 °C elongation at 68 °C for 1 min. Then a final elongation was run at 68 °C for 5 min. The PCR amplicons were observed on 1.5 % agarose gel electrophoresis prepared in 1X TAE stained with red gel (5 mg/ml). The PCR amplicons were purified and sequenced at Macrogen Europe.

2.5. Preliminary screening for antimicrobial activity

Screening of antibacterial activity was done as described by Jayatilake and Munasinghe (2020). Briefly, isolated endophytic fungi were assessed for antimicrobial activity against *E. coli* (ATCC 25822), *B. subtilis*, and *S. aureus* (ATCC 29213) using the Agar plug diffusion method. Endophytic fungi with mycelia plug a disc of 9 mm from seven days old in PDA were inoculated on the surface of nutrient agar seeded with standardized test organisms (0.5 McFarland standard turbidity). The inoculated plates were incubated at 37 °C for 24 h while observing for the appearance of zones of inhibition performed three times. Category 0 (I = 0 mm) which is no inhibition; Category 1, weak inhibition ranges from 0 to less than or equal to 1 mm; Category 2, moderate inhibition which ranges from 1 mm to less than or equal to 3 mm; category 3 strong inhibition which had inhibition above 3 mm), (Zhao et al., 2019).

2.6. Data analyses

BLAST was used to compare the 16 sequences obtained from this study to GenBank Sequences. GenBank sequences were chosen by considering their quality and similarity to the query sequence. To align the sequences, MAFFT version 7 (<https://mafft.cbrc.jp/alignment/server/>) was used, while AliView (Larsson, 2014) was used to edit the sequences manually whenever necessary. The analyses excluded regions that were ambiguously aligned. The data matrix contained 710 bp of unambiguously aligned sites of nucleotide sequences. In MrModeltest 2.3, the Akaike Information Criterion (AIC) was employed to choose the suitable DNA evolution model for the analyses (Nylander, 2004). The GTR plus Invariant Gamma model was used for the ITS region. The MrBayes 3.2.6 software was used for Bayesian inference and posterior probability was used to estimate branch support (PP; Ronquist and Huelsenbeck, 2003). Employing four Markov chains Monte Carlo gnrrate10 million trees, which were sampled every 100 generations, and burned off 25 % of the trees. RAxML v.8.2.10 calculated the maximum likelihood using the General Time Reversible plus G + I site substitution model (Stamatakis, 2014). To obtain branch support, 1,000 replicates of maximum likelihood bootstrapping (ML) were used (Hillis and Bull, 1993). Significant Bayesian PPs of 0.95 (Alfaro et al., 2003) and ML bootstrapping of 70 % were obtained.

2.7. Diversity indices

To determine a dominant taxon amongst isolated endophytes from leaves of *O. latifolia*, the equations below were used to calculate the Shannon-Wiener index (H'), Margalef index (dM), Simpson index (D), and Pielou evenness index (J) (Kusari et al., 2013): Where P_i is the

$$H' = - \sum_{i=1}^S (P_i)(\ln P_i)$$

$$D = - \sum_{i=1}^S (P_i)^2$$

$$dM = - \sum_{i=1}^S (P_i)^2$$

isolates number (N_i) of a specific taxon divided by the total isolates number (N). For a taxon to be considered dominant, P_i should exceed Camargo's index ($1/S$) (Camargo, 1992; Kusari et al., 2013). The number of fungal taxa is represented by S , and H' represents the Shannon-Wiener index, Margalef index (dM), and Simpson index (D),

3. Results

3.1. Endophytic fungi identification and phylogenetic analysis

Sixteen fungi were isolated from *O. latifolia* using PDA media. Morphological features of the mycelia of endophytes isolated varied in texture, growth rates, and pigmentation (Fig. 1). *Nigrospora aurantiaca*, *Phyllosticta capitalensis*, *Nigrospora oryzae*.

The molecular identification was made using the ITS nucleotide sequence. A total of 16 new sequences were generated in this study. Using BLAST search on NCBI, the sequence results that showed the highest similarity based on ITS query sequences were selected for inferring phylogeny (Table 1). The phylogenetic analyses used a sum of 37 sequences from 32 Sordariomycetes species, 3 Dothideomycetes species, and two Arthoniomycetes sequences as out-group. The phylogeny resulting from Bayesian was similar to the one from Maximum likelihood analyses. Only tree topology resulting from Maximum likelihood is presented (Fig. 2). Endophytes isolated in this study were categorized into two classes based on phylogenetic analyses (*Sordariomycetes* and *Dothideomycetes*), five orders (*Xylariales*, *Diaporthales*, *Sordariales*, *Glomerellales*, and *Botryosphaerales*) of subphylum *Pezizomycotina* (Fig. 2). Phylogenetic tree established in this analysis all five orders were well-supported subclade (PP = 1.0, BP = 100). At the species level, fifteen isolates were identified, representing eight confirmed species. Remained one isolate was characterized at the family level. Out of 15 isolates identified, they were classified into four genera; nine isolates were from *Nigrospora*, three *Diaporthe*, two *Colletotrichum*, one *Phyllosticta*, and the other one was identified at the family level. Fig. 3.

The diversity indices of the studied endophytes isolated from leaves of *O. latifolia* showed that the Camargo's index ($1/S$) at the genera and species level were 0.20 and 0.11, respectively. Based these findings, the dominant genus was found to be *Nigrospora* ($P_i = 0.5$), while the dominant species were *Nigrospora oryzae* (25 %, $P = 0.25$), *N. sphaerica* (25 %, $P = 0.25$), and *Colletotrichum karstii* (13 %, $P = 0.125$) (Fig. 2). The Shannon-Wiener index (H'), Simpson index (D) and Margalef index (dM) were 1.99, 0.84 and 2.89 correspondingly.

3.2. Antimicrobial activity screening

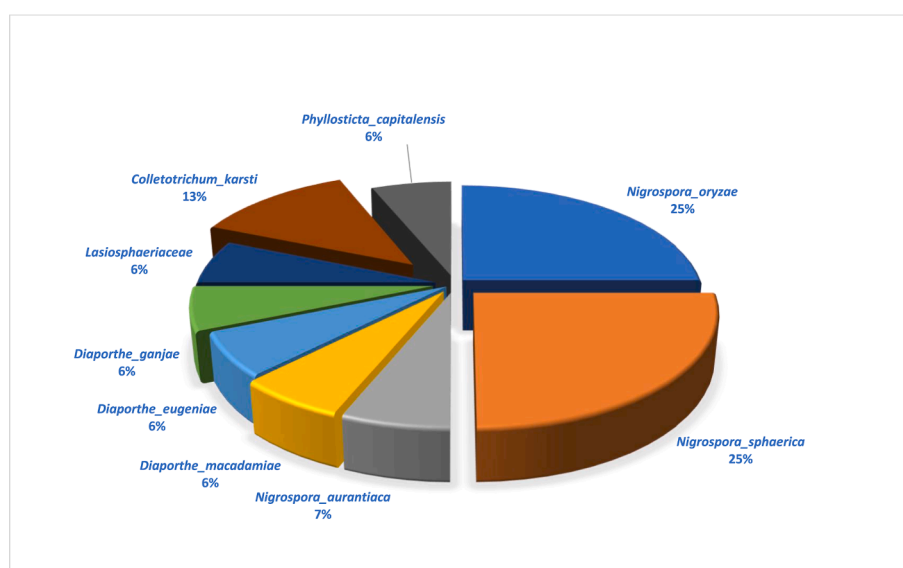
All 16 endophytes were screened for their antibacterial potential. *N. aurantiaca* (14H), *N. oryzae* (7H), and *P. capitalensis* (15) showed potent inhibition, and species of *D. eugeniae* (9H) and *N. oryzae* (1H), *N. oryzae* (2H), *N. oryzae* (3H) had moderate inhibition. On the other hand, two other species of *D. ganjae* (10H), *N. sphaerica* (5H), displayed a weak zone of inhibition. Furthermore, the remaining seven isolates did not show a clear zone of inhibition against tested strains, as presented in Figs. 4 & 5.

4. Discussion

Both classical and molecular techniques were used to characterize isolated endophytic fungi in this study. Macro-morphology features of endophytic fungi varied in terms of colony color and hyphae structure

Table 1Endophytic fungi identification from leaves of *Oxalis latifolia* using molecular marker internal transcribed spacer.

Fungi Endophyte isolate	BLAST results from GenBank	Query cover (%)	Percentage similarity (%)	Accession number
JH 22_1(OQ354870)	<i>Nigrospora_oryzae</i>	98	99.44	KT898587
JH 22_2(OQ354871)	<i>Nigrospora_oryzae</i>	99	99.20	EU821485
JH 22_3(OQ354872)	<i>Nigrospora_oryzae</i>	99	99.79	KT898587
JH 22_4(OQ354873)	<i>Nigrospora_sphaerica</i>	100	100	MT597827
JH 22_5(OQ354874)	<i>Nigrospora_sphaerica</i>	99	99.15	MT597827
JH 22_6(OQ354875)	<i>Diaporthe_macadamiae</i>	100	99.42	NR_168240
JH 22_7(OQ354876)	<i>Nigrospora_oryzae</i>	100	100	MH141277
JH 22_8(OQ354877)	<i>Nigrospora_sphaerica</i>	99	99.21	MT597827
JH 22_9(OQ354878)	<i>Diaporthe_eugeniae</i>	100	98.93	MN121408
JH 22_10(OQ354879)	<i>Diaporthe_ganjae</i>	98	99.02	MK247957
JH 22_11(OQ354880)	<i>Lasio-sphaeriaceae</i>	97	97.85	MK247867
JH 22_12(OQ354881)	<i>Nigrospora_sphaerica</i>	99	99.38	MT597827
JH 22_13(OQ354882)	<i>Colletotrichum_karsti</i>	99	100	MZ724780
JH 22_14(OQ354883)	<i>Nigrospora_aurantiaca</i>	99	100	MN341424
JH 22_15(OQ354884)	<i>Phyllosticta_capitalensis</i>	99	99.98	MN121397
JH 22_16(OQ354885)	<i>Colletotrichum_karstii</i>	100	100	OM436864

**Fig. 2.** Endophytic fungi species composition from the leaves of the medicinal plant *Oxalis latifolia*.

and pigmentation. The color ranged from whitish-cream to orange, grey, and pale Micro-morphological features (Fig. 1). It is true that morphological characteristics, mainly microscopic characters, are critical for fungi identification. However, microscopic identification of endophytic fungi remains challenging as the majority of fungal isolates fail to sporulate in culture media (Cui et al., 2021). As a result, the use of molecular markers such as ITS, among others, is required. The ITS rDNA-constructed phylogenetic tree results shed light on the evolutionary connections between the endophytic fungus under study. Similar tree topologies were obtained by both Bayesian and Maximum Likelihood analyses, suggesting the robustness of the inferred relationships. The results of the phylogenetic analyses classified fungi into two main classes, Sordariomycetes and Dothideomycetes. These classes comprise several orders within the subphylum Pezizomycotina. These classes are common endophytic fungi isolated from *Oxalis* (Mwanga et al., 2019). This broad taxonomic representation suggests a rich fungal community inhabiting the plant host. Furthermore, this study identified endophytic fungi belonging to five different orders; Xylariales, Diaporthales, Sordariales, Glomerellales, and Botryosphaeriales. Within the plant ecology, each order most likely represents unique ecological niches or functional roles (Funk et al., 2017). From our analyses, a total of fifteen isolates were classified into four genera; *Nigrospora*, *Diaporthe*, *Colletotrichum*, and *Phyllosticta*. This diversity of species highlights the complexity of the endophytic fungal community associated with

O. latifolia. While most isolates were identified at the species level, one isolate was characterized only at the family level (*Lasio-sphaeriaceae*). This implies that the species with an unknown identity most likely belongs to a new taxon and further taxonomic characterization or molecular analysis may be necessary to assign species-level identification accurately. These findings underscore the importance of understanding the composition and diversity of endophytic fungal communities within medicinal plant ecosystems. The findings have implications for ecological studies, bioprospecting for novel bioactive compounds, and agricultural applications such as biocontrol and plant growth promotion.

Traditionally, *O. latifolia* is used as an appetizer, as a treatment for dysentery and diarrheas, and as a remedy for skin diseases. The leaves are known to be anti-inflammatory, refrigerant, and antiscorbutic (Krishnan & Murugesu, 2019). Antibacterial activity testing is critical since their natural compounds have tremendous effectiveness against various pathogens (Deshmukh et al., 2018). In this study, 16 endophyte isolates were assessed for antibacterial activity against *B. subtilis*, *E. coli*, and *S. aureus*. Three isolates, *N. aurantiaca* (14H), *N. oryzae* (7H) and *P. capitalensis* (15), showed strong potent inhibition against organisms tested (Fig. 5). This study's findings are in synchronicity with previous reports, for example, Safwan et al., (2021) reported bioactive compounds from *N. aurantiaca* with inhibitory effects against bacteria. Similarly, crude extracts from *P. capitalensis* inhibited growth against *B. cereus*, *E. coli*, and *P. aeruginosa* (Wikee et al., 2013). Also, Rathod

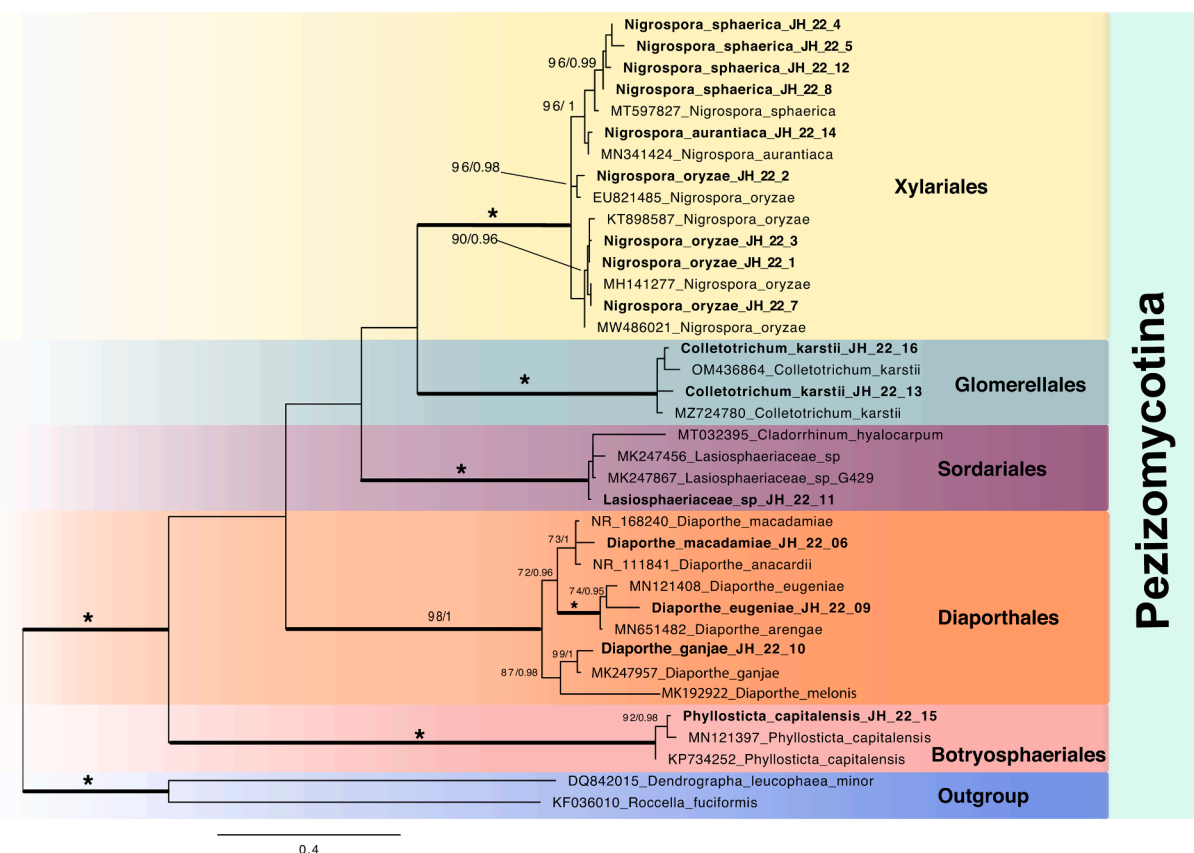


Fig. 3. Phylogenetic relationships derived from Bayesian and Maximum likelihood analyses of concatenated ITS datasets. Two *Arthoniomycetes* species were used to root the tree. Each internal branch's two support values represents the PPs and MLbs proportions, accordingly. The highlighted indicates sequences produced in this study. An asterisk signifies that this node has MLbs = 100 and PP = 1.0 support.

et al. (2014) reported griseofulvin produced by *N. oryzae* with antimicrobial activity against human pathogens. From this study observed growth inhibition was moderate *D. eugeniae* (9H) and *N. oryzae* (1H), *N. oryzae* (2H), and *N. oryzae* (3H) (Fig. 5). Likewise, *Diaporthe* and *Nigrospora* have previously been reported to produce compounds with selective antibacterial activity (de Carvalho et al., 2021). This study also observed that *C. karstii*, *D. macadamiae*, *Lasiosphaeriaceae* sp., and *N. sphaerica* did not inhibit growth against tested organisms (Fig. 5). In contrast, Wu et al. (2018) previously reported high antibacterial activities of *N. sphaerica* extract. Although the antimicrobial screening of *C. karstii* and *N. sphaerica* in this study did not show bacterial growth inhibition against tested pathogens, extracts from *C. karstii*, and *N. sphaerica* have previously been found to have anti-inflammatory, anticancer, immune-modulatory, and α -glucosidase inhibitory activities (Ukwatta et al., 2019). Hence, additional research is required to investigate other beneficial bioactivities of isolated endophytic fungi besides antimicrobial activity.

According to diversity indices, Camargo's index ($1/S$) was 0.20 at the genera level and 0.11 at the species level. Because a taxon is considered dominant if $P_i > \text{Camargo's index}$ (Camargo, 1992; Kusari et al., 2013), the dominant genus was *Nigrospora* ($P_i = 0.5$), with the dominant species being *Nigrospora oryzae* ($P_i = 0.25$), *N. sphaerica* ($P_i = 0.25$), and *Colletotrichum karstii* ($P_i = 0.125$). The current study's species diversity indices showed a higher Shannon-Wiener index (H') of 1.99, Simpson index of (D) of 0.84, and Margalef index (dM) of 2.89. These findings imply that the studied medicinal plants have a higher endophytic fungi species diversity, supported by the higher Shannon-Wiener index (H') of 1.99. In general, the greater the Shannon-Wiener (mostly ranges 1.5 and 4.5) the more diverse the species are found in the habitat. The higher the D , the more hereditary variation there is and the higher the chance to acclimatize to micro-ecological variations (Li et al., 2016). Similarly, the

Margalef index (dM) reflects the abundance of fungi species, as the larger the dM , signifies a higher abundance of the endophytic fungi. (Li et al., 2016). The observed significant diversity of endophytic fungi suggests that leaves of *O. latifolia* host a rich variety of fungal species, which could have implications for their ecological roles and potential applications in various fields such as pharmaceuticals and agriculture.

5. Conclusion

This study aimed to explore the diversity of endophytic fungi found in *O. latifolia*. In this study, 16 isolates of endophytic fungi from leaves of *O. latifolia* were identified by conventional methods and ascertained by molecular marker and phylogenetic analysis. *Nigrospora oryzae*, *N. sphaerica*, and *C. karstii* were found to be the dominant species. Findings from antimicrobial screening suggest that the studied isolated endophytic fungi possess antimicrobial activities, worth further exploration for their potential in discovering novel bioactive compounds suitable for pharmaceutical, and agro-industrial applications. Subsequent research could involve scaling up metabolite production, fractionating crude extracts to identify the active fraction, and further characterizing the bioactive compounds present. The notable diversity of endophytic fungi observed implies that the leaves of *O. latifolia* accommodate a diverse array of fungal species. This finding holds potential implications for their ecological functions and possible utilization in sectors like pharmaceuticals and agriculture. Additionally, the identification of dominant fungal genera and species highlights the importance of understanding the composition and dynamics of endophytic fungal communities within medicinal plant ecosystems.

Disclosure of Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

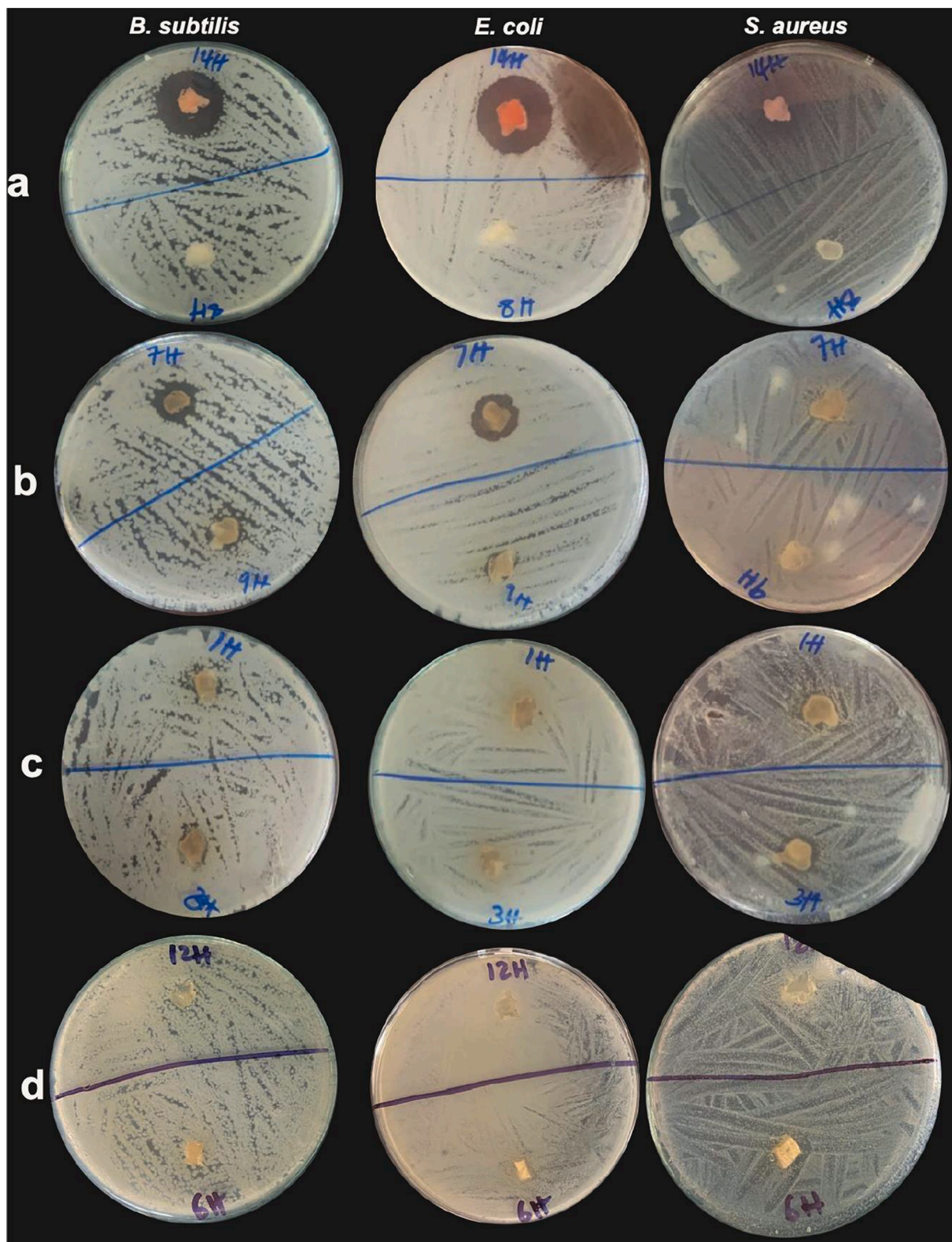


Fig. 4. Zone of inhibition on dual culture of the isolated endophytes against *B. subtilis*, *E. coli*, and *S. aureus* (a) strong of inhibition for *Nigrospora aurantiaca* (14H) all tested pathogens but no clear zone of inhibition for *Nigrospora sphaerica* (8H) (b) Strong zone of inhibition of *Nigrospora oryzae* (7H) on both *B. subtilis* and *E. coli* but not *S. aureus* while moderate zone of inhibition for *Diaporthe eugeniae* (9H) on both *B. subtilis* and *E. coli* but not *S. aureus* (c) Moderate zone of inhibition of *Nigrospora oryzae* (3H) and *N. oryzae* (1H) on both *B. subtilis* and *S. aureus* but not while no clear zone of inhibition for *E. coli* (d) Both *Nigrospora sphaerica* (12) and *Diaporthe macadamiae* (6) did not show a clear zone of inhibition on all tested organism.

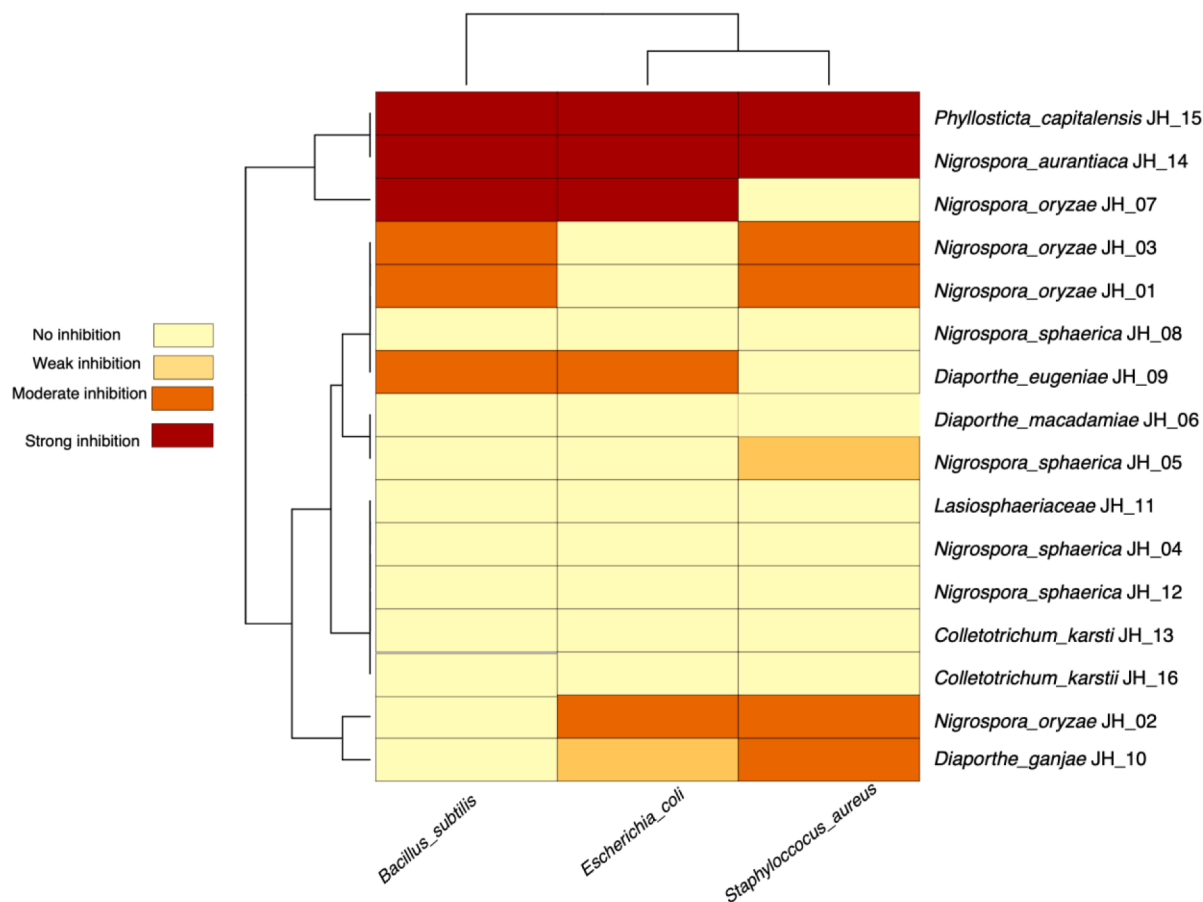


Fig. 5. Zone of inhibition against the tested pathogenic bacteria of isolated endophytic fungi displaying a range of activity from Strong to weak.

CRedit authorship contribution statement

J.M. Hussein: Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Visualization, Investigation, Validation, Formal analysis, Methodology, Resources, Project administration, Software. **H. Myovela:** Conceptualization, Data curation, Writing – review & editing, Visualization, Investigation, Validation, Formal analysis, Methodology. **D.D. Tibuhwa:** Conceptualization, Data curation, Writing – review & editing, Visualization, Investigation, Validation, Formal analysis, Methodology, Resources, Supervision, Project administration, Software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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