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(RESEARCH ARTICLE)



Isolation, identification, and evaluation of biological activities of *Daldinia eschscholtzii*, an endophytic fungus isolated from the leaves of *Musa paradisiaca*

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Abstract

Fungal endophytes of medicinal plants are gaining interest in the prospecting for biorelevant molecules due to their capacity to contribute novel compounds. This study was designed to identify and evaluate secondary metabolites of *Daldinia eschscholtzii* isolated from leaves of *Musa paradisiaca* for biorelevance. Plant leaves collection, fungal isolation, molecular identification, fermentation, and extraction of secondary metabolites were carried out following standard procedures. The crude extract was tested for antimicrobial, antioxidant, cytotoxic and antiviral properties; and further analyzed for their phyto-constituents using High performance liquid chromatography – diode array detection (HPLC-DAD). At 1000 µg/ml, *D. eschscholtzii* extract exhibited antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* producing IZD of 3 mm, 5 mm, and 7 mm respectively, but showed no antifungal activity. It showed antioxidant activity in the DPPH free radical scavenging assay producing an inhibition of 65.9% at a concentration of 500 µg/mL. At a concentration of 10 µg/mL, the *D. eschscholtzii* extract showed weak cytotoxic activity against L5178Y mouse lymphoma cells with growth inhibitions of 17.4% but showed a robust inhibition of HIV-1 reverse transcriptase activity of 97 % at 250 µg/mL. HPLC analysis of the extract/fraction revealed the presence of four bioactive compounds: aureonitol, pestalotiolactone A, cyclophenol, and palitantin. The detection of these metabolites further confirms the potentials of these endophytes as possible sources of bioactive molecules.

Keywords: Endophytic fungi; *Daldinia eschscholtzii*; *Musa paradisiaca*; Secondary metabolites; Reverse transcriptase; 1,1-diphenyl-2-picryl-hydrazyl (DPPH).

1. Introduction

Various infectious diseases threaten human health worldwide [1,2]. Endophytes are microorganisms living in the intercellular spaces of healthy host tissues without causing any obvious symptoms, while synthesizing metabolites believed to be of importance in novel drug molecules bioprospecting. This could be key to solving the many infectious diseases threatening mankind till date. Endophytic microorganisms are fundamental for the discovery of new substances for human therapeutics including antibiotics, anti-malarials, anticancer [3-5] and host of other sicknesses.

In comparison to other natural sources like plants, microorganisms are highly diverse but narrowly explored. Studies based on estimation of microbial populations have revealed that only about 1 % of bacteria and 5 % of fungi have been characterized and the rest remain unexplored for their contribution to the human welfare. In addition, more than 60 %

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of the anticancer and 70 % of the antimicrobial drugs currently in clinical use are natural products or natural product derivatives [6].

Endophytes are relatively not well studied and are assumed to be a possible source of important and novel natural products [7]. The resistance of pathogenic microorganisms to drugs and antibiotics has become a major challenge in the health sector leading to reduction in drug effectiveness and economic wastage. This challenge has stirred up the research for new and more effective drug molecules.

Musa paradisiaca. (Banana, common name) is a treelike herb of the genus *Musa* belonging to the family Musaceae which grown extensively in all tropical countries (including Nigeria). Bananas are an excellent source of potassium. Potassium can be found in a variety of fruits, vegetables, and even meats, however, a single banana provides you with 23 % of the potassium that you need daily [8]. *M. paradisiaca* (Banana), is one of the most common and most important fruit trees grown in Nigeria, as well as, globally due to their high nutritional and medicinal value [8].

Banana fruits (Musaceae) possess high amounts of bioactive compounds which can influence human health. These include: Potassium, Fiber, Calcium, Magnesium, Phosphorus, Selenium, Iron, Vitamins A, B₂, B₆, C, E, Niacin, Folate, and Pantothenic Acid [8]. Catecholamines, amino acids, Alkaloids, Carbohydrates, vitamins etc. Its health promoting properties are as follows: improves kidney health [8], possess antioxidant properties [8], antibacterial properties, Nerve calming properties due to rich vitamin B [8]. Serotonin, tryptophan, nor-epinephrine, iron, crystallizable and non-crystallizable sugars, vitamin C, vitamins B, albuminoids, fats, mineral salts Acyl steryl-glycosides sitoindoside-I, sitoindoside-II, sitoindoside-III, sitoindoside-IV [9, 10].

Previous studies have demonstrated the huge potentials possessed by secondary metabolites expressing endophytes from Nigerian plants, with several interesting bioactivities being recorded [11-16]. With Nigeria's rich medicinal plant varieties, and the increasing cases of resistance, there is need to explore these plant varieties with reference to their endophytic microbial communities, for new biorelevant molecules.

2. Material and methods

2.1. Collection of plant materials

Fresh, matured and healthy banana leaves were collected randomly from three (3) banana trees in the botanical garden of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu, (6° 8'35"N, 7° 2'8" E) Anambra state (South-Eastern Nigeria) in the month August 2016 and were authenticated by a plant taxonomist Mrs. Onwunyili Amaka, and voucher number PCG/474/A/065 assigned. The voucher specimen was deposited at the Pharmaceutical Science Faculty Herbarium (6° 8'35"N, 7° 2'8" E).

2.2. Isolation of endophytic fungi from plant leaves

Isolation of endophytic fungus from leaves of *M. paradisiaca* was carried out aseptically in a laminar flow chamber as previously described [17]. The isolation of endophytic fungi. Healthy mango leaves were surface washed in running water to remove dust and debris. After washing, the leaf samples were cut into segments (about 1-2 mm²) and were surface-sterilized further by washing for 2 min in 70% ethanol, 2% sodium hypochlorite (NaOCl) for 2-3 min and then for 2 min in 70 % ethanol, then followed by rinsing in sterile distilled water. The samples were blotted on sterile filter paper [17]. About four (4) segments from each plant were selected and placed in each Petri dish containing Malt Extract Agar (MEA) supplemented with chloramphenicol 50 mg/L. Sterilized segments were pressed on to the surface of the MEA medium for confirmation. This was done in triplicate. Absence of visible fungal growth on the growth medium confirmed surface sterilization to be effective [18]. The plates were sealed and incubated at 27 ± 2 °C for 7 days. Fungal growth from the segments were periodically checked for, and hyphal presence from the leaf segments were sub-cultured to obtain pure colonies.

2.3. Identification of isolated fungi

Taxonomic identification of the endophytic fungus was achieved by DNA amplification, sequencing of the fungal internally transcribed spacer (ITS) regions [19].

2.3.1. DNA extraction and Amplification

DNA was extracted from culture of endophytic fungi mycelia using a ZR Fungal/Bacterial DNA Miniprep™ Kit from Zymo Research Corp, USA according to the manufacturer's protocols. Polymerase Chain reaction (PCR) was then conducted to amplify the internal transcribed spacer (ITS) region of the extracted DNA, including the 5.8s rDNA, using the primers ITS1 and ITS4 under the following conditions: Initial denaturation at 90 °C for 15 min; denaturation at 95 °C for 1 min; annealing at 56 °C for 1 min and extension at 72 °C for 1 min. The PCR process lasted a total of 35 cycles and ended with the extension at 72 °C for 10 minutes.

2.3.2. Sequencing

The PCR products were sequenced and the base sequences were compared to the data in Gen Bank. Blast search of the sequences, to identify the DNA sequence of the fungi, was performed following the link: http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=NucleotidesPROGRAM=blastnPAGE_TYPE=BlastSearchBlast_SPEC=

2.4. Fermentation of endophytic fungi, extraction and isolation of metabolites

Solid state fermentation was carried out by growing the fungus in 1 L Erlenmeyer flasks containing sterile solid rice medium (100 g of rice + 100 mL of distilled water, autoclaved at 121 °C at 15 psi for 1 h) under static conditions at 22 °C for 21 days. At the end of fermentation, the fungal cultures were extracted with ethyl acetate and the crude extract concentrated under reduced pressure. The corresponding extracts were weighed, and their respective percentage yields recorded in milligram.

2.5. Chromatographic analysis

2.5.1. Analytical High-Performance Liquid Chromatography (HPLC)

The HPLC analysis of the fungal crude extract was carried out as reported by Eze et al. (2018) [16]. Analytical HPLC Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S, Dionex Softron GmbH, Germering, Germany) was used to identify important peaks in the extract and fractions, as well as to evaluate the purity of isolated compounds. Additionally, known substances could be identified by comparison of the obtained UV spectra with the internal UV-spectra library using the online software. For the routine analytical HPLC detection, a solvent system of nanopure water adjusted to pH 2.0 by addition of phosphoric acid (eluent A) and MeOH (eluent B) with a flow rate of 1 mL/min employing the standard gradient system was used.

2.5.2. Size Exclusion Chromatography

A weight of 260 mg of the fungal extract was subjected to size exclusion chromatography using Sephadex LH-20 (Sigma-Aldrich, Germany) as the stationary phase. Elution was performed using methanol (MeOH) (100 % v/v) as the mobile phase. The components of the extract were separated and distributed on the stationary phase according to their molecular size.

2.5.3. Semi Preparative High-Performance Liquid Chromatography (SP-HPLC)

This technique was used for isolation and purification of compounds from fractions pre-purified using size exclusion chromatographic separation. A metabolite-containing fraction (BAL4-5) was further purified by semi-preparative HPLC. The mobile phase consisted of methanol and nanopure water. Each injection consisted of 1–3 mg of the fraction dissolved in 100 µl of the solvent system. The solvent system was pumped through the column at a rate of 5 mL/min. The eluted peaks were detected by the online UV detector and collected separately in clean test tubes. The compounds: aureonitol and palitantin were isolated from fraction (BAL4-5) using semi-preparative HPLC.

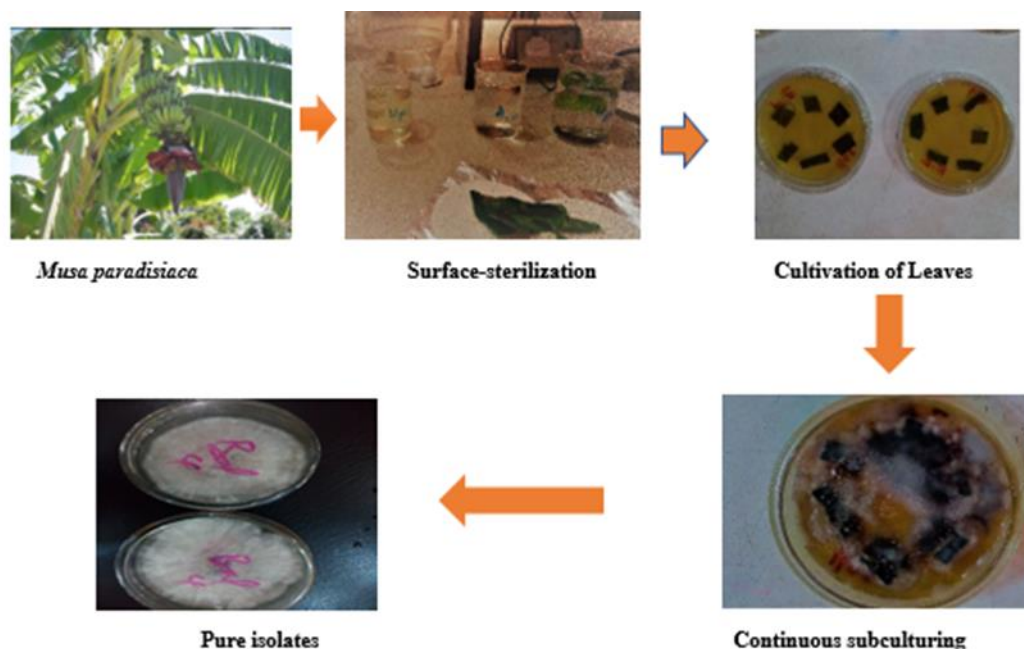


Figure 1: Isolation of endophytic fungi from *Musa paradisiaca*

2.6. Antimicrobial (bacteria and fungi) Assay

Antimicrobial screening of the crude fungal extracts was carried out using the agar well diffusion assay method as previously described by Akpotu et al. (2017) [20]. A concentration of 1 mg/mL of the fungal extracts was prepared by dissolving the extracts in dimethyl Sulphoxide (DMSO). A range of concentration between 1 mg/mL and 12.5 µg/mL were obtained by two-fold serial dilutions. Standardized broth cultures (0.5 McFarland turbidity standard) suspension of each of the test isolates of test bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*) and fungal strains (*Aspergillus niger* and *Candida albicans*) were spread aseptically onto the surface of Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates respectively by using sterile cotton swabs. All culture plates were allowed to dry for about 5 min and agar wells were made by using a sterile cork-borer (6 mm in diameter). These wells were respectively filled with 20 µL of the fungal extracts and controls. The plates were then kept at room temperature for 1 h to allow the agents to diffuse into the agar medium. Ciprofloxacin (5 µg/mL) and miconazole (50 µg/mL) were used as positive controls in the antibacterial and antifungal evaluations respectively; while DMSO was used as the negative control. The MHA plates were then incubated at 37 °C for 24 h, and the SDA plates were incubated at 25-27 °C for 2-3 days. The inhibition zones diameters (IZDs) were measured and recorded. The size of the cork borer (6 mm) was deducted from the values recorded for the IZDs to get the actual diameter. This procedure was conducted in triplicate and the mean IZDs calculated and recorded.

2.7. Determination of Minimum Inhibitory Concentrations (MIC)

The minimum inhibitory concentration (MICs) of the extract was determined for each of the test organisms that were susceptible to the extract during the antimicrobial screening using the agar dilution method as described by Akpotu et al. (2017) [20]. A stock solution of 10 mg/ml of the extract dilutions was prepared, thereafter two-fold serial-dilution was done to obtain graded concentrations such as these (5, 2.5, 1.25, and 0.625 mg/mL). Then, ten-fold dilution was made for each of the concentrations. Here, 1 mL from each of this concentration is transferred into sterile Petri dishes, and 9 mL of molten agar cooled to 40-50 °C was added to it and rocked clockwise and anticlockwise to ensure proper mixing. Thereafter, a loopful of each of the test organisms previously standardized to 0.5 McFarland turbidity (1.5×10^8) was streaked on the surface of the solidified growth media. The addition of the agar (i.e. specific volume) dilutes the concentration of the extract to a final concentration of 1, 0.5, 0.25, 0.125 and 0.0625 mg/mL. Also, for the negative control, the organisms were streaked on the sterile molten agar that does not contain the extract but 1 mL of the diluents DMSO. The culture plates were then incubated at 37 °C for 24 h. After incubation, the plates were examined for microbial growth by checking for growth using a plus sign (+) indicating growth while a negative sign (-) indicates no growth. The minimum dilution (concentration) of the extracts completely inhibiting the growth of each organism was taken as the MIC. This procedure was conducted in triplicate.

2.8. Determination of Minimum Bactericidal Concentrations (MBCs)

The MBC of the fungal extract was determined by a method previously described [20]. This is the minimal concentration which kills off all the cell in a microbial population. It is an extension of the MIC procedure (stated above) carried out. In the MBC, the plates without visible growth in the MIC test are selected and incubated for 48 more hours. Thereafter, the plates are checked for proof of growth. The MBC is the lowest concentration producing no evidence growth.

2.9. Cytotoxicity assay

Tetrazolium MTT (3, [4,5- dimethylthiazolyl-2]-2,5- diphenyl tetrazolium bromide assay was performed following the method described by Carmichael et al. [21] and % cell viability was deduced by spectrophotometric determination of accumulated formazan derivative in treated cells at 560 nm in comparison to control cells. Eagle's minimal essential medium supplement with 10 % horse serum was used to grow the L5178Y mouse lymphoma cells. Penicillin (100 units/mL) and streptomycin (100 units/mL) were added to the medium. The cells were maintained in a humidified atmosphere at 37 °C with 5 % CO₂. Of the test samples to be analyzed in the bioassay, stock solutions of crude extracts (10 µg/mL) in ethanol (96 % v/v) were prepared. Optimally replicating cells were harvested, counted, and diluted appropriately. Of the cell suspension, 50 µL containing 3750 cells were pipetted into 96 - well microtiter plates. Subsequently, 50 µL of a solution of the test samples containing the appropriate concentration was added to each well. The test plates were incubated at 37 °C with 5 % CO₂ for 72 h. A solution of MTT was prepared at 5 µg/mL in phosphate-buffered saline (PBS; 1.5 mM KH₂PO₄, 6.5 mM Na₂HPO₄, 137 mM NaCl, 2.7 mM KCl; pH 7.4) and from this solution, 20 µL were pipetted into each well. The yellow MTT penetrated the cells and in the presence of mitochondrial dehydrogenases, MTT was transformed into its blue formazan complex. After incubating for 3 h 45 min at 37 °C in a humidified incubator with 5 % CO₂, the medium was centrifuged (15 min at 210 x g) with 200 µL DMSO and lysed to liberate the formazan product. Prior to the absorbance reading at 520 nm, the mixture was shaken thoroughly. The colour intensity could be correlated with the number of healthy living cells and cell survival was calculated using the formula:

$$(\text{Absorbance of untreated cells} - \text{Absorbance of culture medium}) / (\text{Absorbance of treated cell} - \text{Absorbance of culture medium}) \times 100$$

All experiments were carried out in triplicate. As negative controls, media with 0.1 % (v/v) ethanol were included in all experiments.

2.10. Antioxidant assay

The free radical scavenging activity of all the extract and compounds was evaluated by 1, 1-diphenyl-2 picryl-hydrazyl (DPPH) according to the method by Shen et al. [22] with modification. A volume of 50 mL of 0.2 mM solution of DPPH in methanol was prepared by weighing 3.94 mg of DPPH to 50 mL of methanol. Also, 2 mL of 0.2 mM solution of DPPH was added to 2 ml of the sample and quercetin respectively and dissolved in methanol (1 mg/mL, 1000 µg/mL). These final reaction mixtures result in a 2-fold dilution of both the extract and DPPH concentrations bringing them to a final concentration of 0.1 mM for the DPPH solution and 500 µg/ml for the samples. The absorbance readings were measured at 517 nm using a UV-VIS spectrophotometer after thorough shaking and standing at room temperature for 30 min. The lower the values of absorbance readings of the reaction mixture, the higher free radical mopping property. The mopping property was calculated by using the following formula:

$$\text{DPPH scavenging effect (\% inhibition)} = (\text{Abs of Blank (A}_0) - \text{Abs of sample (A}_1) / (\text{Abs of blank (A}_0) \times 100/1$$

Where, A₀ is the absorbance of the control and A₁ is the absorbance in the presence of all of the extract samples and reference. All the tests were performed in triplicates. The mean value and standard deviation were determined and recorded.

2.11. HIV-1 reverse transcriptase inhibition assay

This technique is used for the determination of retroviral reverse transcriptase activity by incorporation of digoxigenin- and biotin-labelled dUTP into DNA. HIV-1-reverse transcriptase (4-6 ng) were diluted in 20 µl of lysis buffer in a separate reaction tube. A lysis buffer with no HIV-1-RT was used as a negative control. A 20 µl of the Reverse Transcriptase Inhibitor (RTI) diluted in lysis buffer and 20 µl of reaction mixture per reaction tube was incubated for 1 h at 37 °C. Foil bags was opened for the numbers of Microplate (MP) modules to be used and put into the frame in the correct orientation (the correct fittings would ensure tight support of the MP module). A 60 µl of the extract was transferred into micro-titer plate well of the Microplate modules and covered with foil and incubated for 1 h at 37 °C. After incubation, the solution was discarded completely and rinsed 5 times with 250 µl of washing buffer per well for

30 sec each, washing buffer carefully discarded. The 200 µl of anti-DLG-POD working dilution was added per well and the MP module covered with foil and incubated for 1h at 37 °C. Again, the solution is carefully discarded completely and rinsed 5 times with 250 µl washing buffer for 30 secs and washing buffer carefully and completely discarded. A 200 µl of ABTS substrate solution was added per well and incubate for 20 min at 115-125 °C until color development (green color) was sufficient for photometric detection. The microplates were tapped gently at the sides to ensure homogenous distribution of the colored reaction mixture; and using ELISA reader, the absorbance of the samples was measured at 485 nm (reference wavelength) ~ 490 nm. The resulting signal intensity is proportional to the actual reverse transcriptase activity. To compare the inhibitory activity of the reverse transcriptase inhibitor, the percentage inhibition as compare to a sample that does not contain an inhibitor was calculated as shown below:

$$(BA - BC)/BA \times 100$$

Where: A = Blank, B = Negative control, C = Positive control. Measurement for each sample was carried out in triplicates.

2.12. Statistical Analysis

Data are presented as mean ± standard deviation (SD) of triplicate analysis. Analysis of variance (one-way ANOVA) was used to check the significant mean difference and was achieved using SPSS 20. A probability value of $P \leq 0.05$ was considered to denote a statistical significance difference from their respective controls.

3. Results

The isolated fungal endophyte was identified by molecular characterization to be *Daldinia eschscholtzii* (Table 1).

Table 1 Nucleotide Base Sequence confirming the fungus as *Daldinia eschscholtzii* (Molecular Identification Result).

Code/ Source	DNA Sequence (FASTA format)	Name of fungus
BAL4	CTTACCGCCGTTGCCTCGGCGGGCCGCGTTCGCCCTGTAGTTTACTACCTGGCGGC GCGCTACAGGCCCGCCGGTGGACTGCTAAACTCTGTTATATATACGTATCTCTGA ATGCTTCAACTTAATAAGTTAAAACCTTCAACAACGGATCTCTTGGTTCTGGCAT CGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA ATCATCGAATCTTTGAACGCACATTGCGCCATTAGTATTCTAGTGGGCATGCCT GTTTCGAGCGTCATTTCAACCCTTAAGCCCCTGTTGCTTAGCGTTGGGAATCTAGG TCTCCAGGGCCTAGTTCCCCAAAGTCATCGGCGGAGTCGGAGCGTACTCTCAGCG TAGTAATACCATTCTCGCTTTTGCAGTAGCCCCGGCGGCTTGCCGTAACCCCT ATGTCTTTAGTGGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAAGC	<i>Daldinia eschscholtzii</i>

The results of the antimicrobial screening revealed that at a concentration of 1 mg/ml, the crude extract of *D. eschscholtzii* from *Musa paradisiaca* showed antibacterial activity against *B subtilis*, *P. aeruginosa* and *E. coli* with an inhibition zonal diameter (IZD) of 3 mm, 5 mm and 7 mm respectively but showed no activity against other bacteria (see table 2). The crude extract of *Aspergillus sp* showed no antifungal activity against *A. niger* and *C. albicans*, (See Table 2). At some varied concentrations (10, 5, 2.5, 1.25, 0.625 mg/ml) in a separate experiment, the crude extract of *Daldinia eschscholtzii* showed activity against a resistant (Extended Spectrum Beta Lactamase, ESBL) strain of *E. coli* at 1 mg/ml with an IZD of 4 mm, 4 mm, 4 mm, 3 mm and 0 mm respectively but showed no activity against vancomycin resistant *Staphylococcus aureus* (VRSA) at the same concentration. (See Table 3).

Table 2 Antimicrobial Assay of *Daldinia eschscholtzii* crude extract, showing the Mean Inhibition Zone Diameters (IZDs)(mm) produced against test organisms at 1 mg/ml

EXTRACTS	IZD mm					
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>A. niger</i>	<i>C. albicans</i>
<i>Daldinia eschscholtzii</i>	-	3±0.00b	5±0.00b	7±0.00b	-	-
*CIP(5µg/ml),					10±0.00	8±0.00
*MCA (10 µg/ml)	6±0.00	10±0.00c	7±0.00c	9±0.00c		
*DMSO (100%)	-	0±0.00a	0±0.00a	0±0.00a	-	-
F-Value	-	600.200***	422.866***	2139.382***	-	-

*Ciprofloxacin (CIP) and Miconazole (MCA) are positive controls. *DMSO is negative control. Means within a product followed by the same letter do not differ significantly at $p = 0.05$ (Duncan's test); *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. Number of replicates: 3.

Table 3 Result of Antimicrobial Activity of Crude Extracts of *Daldinia eschscholtzii* against resistant organisms (ESBL producing *E. coli* and Vancomycin Resistant *S. aureus*) at 1 mg/mL.

Extracts	Concentrations	Mean ±SD IZD (mm) against ESBL producing <i>Escherichia coli</i>	Mean ±SD IZD (mm) against Vancomycin Resistant <i>Staphylococcus aureus</i>
<i>Daldinia eschscholtzii</i>	10 mg/ml	4.6±0.33d	-
	5 mg/ml	3.6±0.33b, c	-
	2.5 mg/ml	3.0±0.10c	-
	1.25 mg/ml	2.0±0.50b	-
	0.625 mg/ml	0±0.00a	-
*Ciprofloxacin (CIP)	5 µg/ml	10.0±0.00e	-
*DMSO (NC)	100%	0±0.00a	-
F-value		74.594***	-

*DMSO is negative control. *Ciprofloxacin (CIP) is positive control. analysis. Means within a product followed by the same letter do not differ significantly at $p = 0.05$ (Duncan's test); *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. Number of replicates: 3.

The *Daldinia eschscholtzii* extract also showed a weak cytotoxic activity in an MTT (cell proliferation assay). At 10 µg/ml, the extract of *Daldinia eschscholtzii* had an 17.4 % cancer cell growth inhibition against L5178Y mouse lymphoma cells.

The *D. eschscholtzii* extract also showed antioxidant activities in the DPPH (free radical scavenging) assay with 65.9 % inhibition at 500 µg/ml.

At 250 µg/ml concentrations, the *D. eschscholtzii* extract also showed a very robust HIV-1 reverse transcriptase inhibition activity of 97 %.

HPLC-DAD analysis detected four secondary metabolites in the extract/fraction of *D. eschscholtzii*: pestalotiolactone A, cyclophenol, aureonitol and palitantin. The structures, Ultraviolet (UV) spectra and chromatograph of the compounds are shown in Figure 2 – 4.

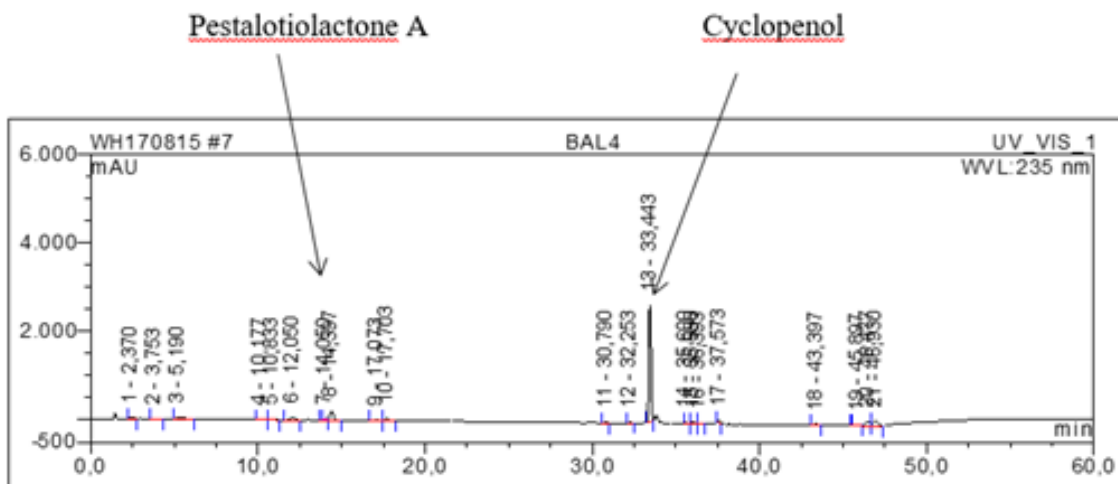
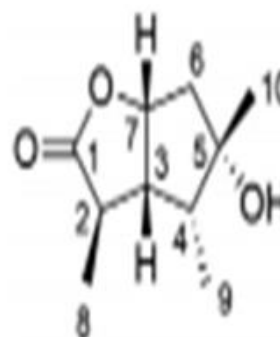
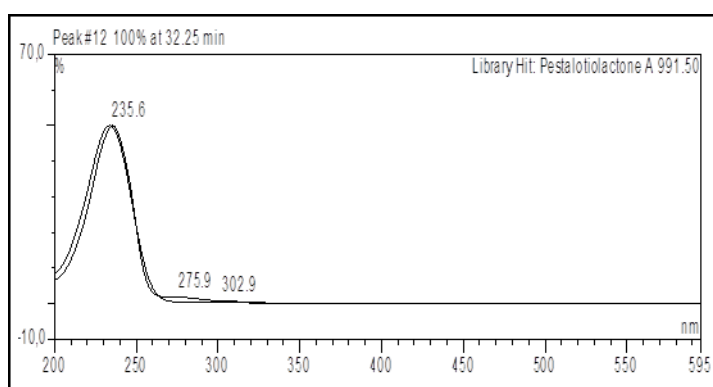
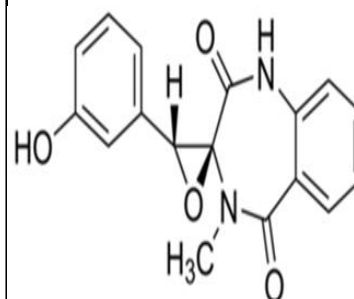
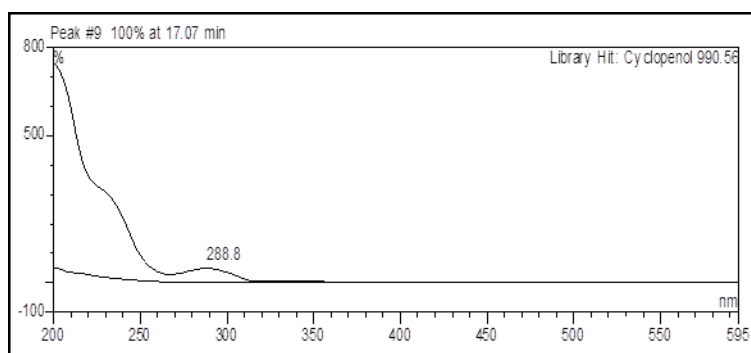


Figure 2 HPLC Chromatograms of *D. eschscholtzii* crude extract showing compounds detected.



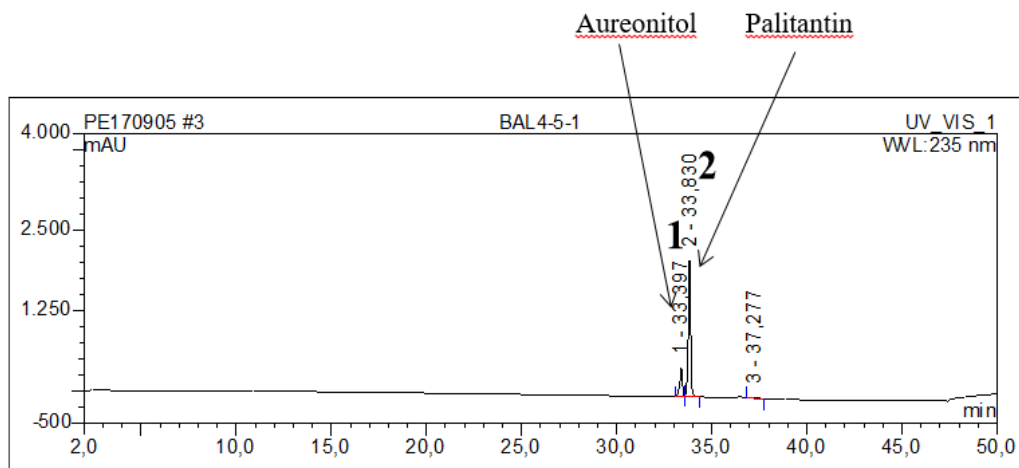
Pestalotiolactone A, $C_{10}H_{16}O_3$, 186g/mol



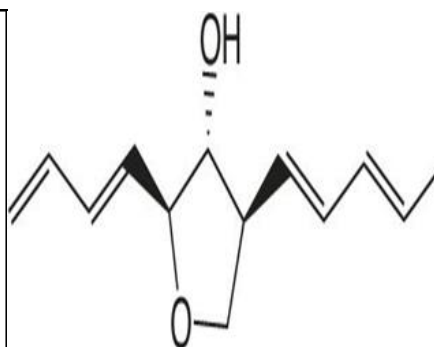
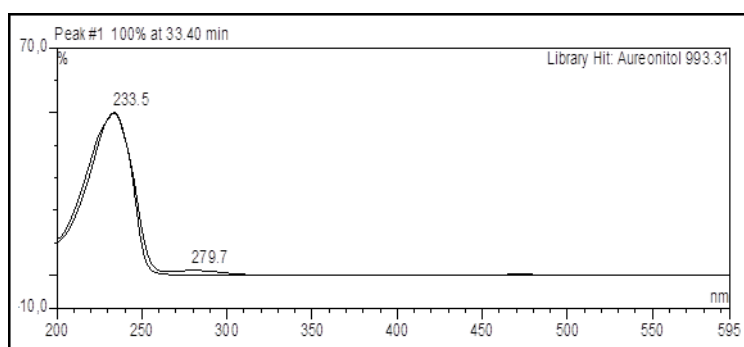
Cyclophenol, $C_{17}H_{14}O_4N_2$, 310.309g/mol

Figure 3 UV Spectra and chemical structures of detected bioactive compounds from crude extract:

Pestalotiolactone and Cyclophenol

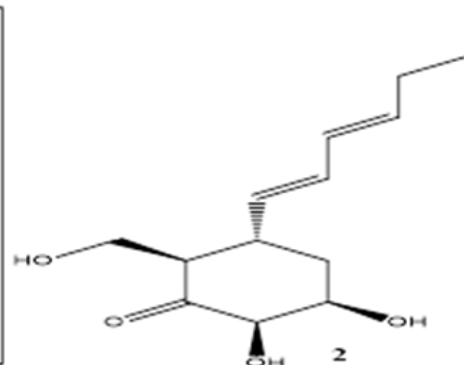
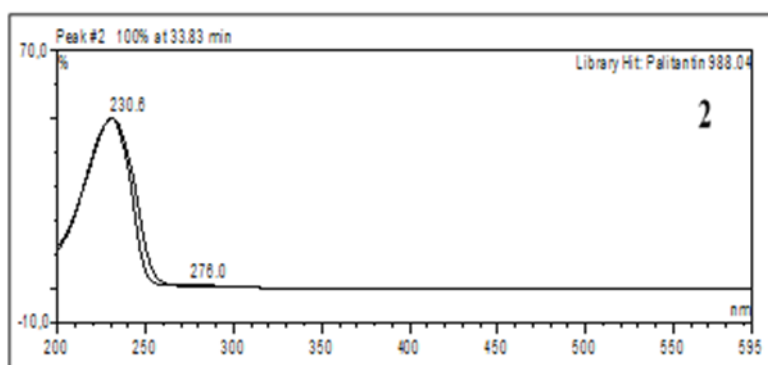


(1)



Aureonitol, $C_{13}H_{18}O_2$; 206.285 g/mol; λ_{max} (nm) = 233.5, 279.7

(2)



Palitantin $C_{14}H_{22}O_4$; 254.32 g/mol; λ_{max} (nm) = 230.6, 276.0

Figure 4 HPLC Chromatograms, UV Spectra and chemical structures of detected bioactive compounds from the fraction of *D. eschscholtzii* fraction showing detected pure compounds

4. Discussion

D. eschscholtzii is a wood-inhabiting endophyte or wood-decaying fungus that is widespread in warm tropical climate [23]. It is characterized by colonies that are white to smoky grey with a slight olivaceous-tone, and by conidiogenous structures with a nodulisporium-like branching pattern [23, 24]. *D. eschscholtzii* grows preferentially on dead or decaying wood substrates and is commonly isolated from dead woody plants such as dicotyledonous crop plants, trees, and occasionally, marine algae [25, 26, 27]. Zhang et al., [27] reported that secondary metabolites of *D. eschscholtzii* possessed an unprecedented immunosuppressive property. Compelling data in the last decade has demonstrated the presence of a wide array of secondary metabolites in this fungus, such as 1,10-binaphthalene-4,40-5,50-tetrol (BNT) (a polyketide derived from 1,8-dihydroxynaphthalene biosynthesis), Cytochalasins (metabolites of mixed polyketide/NRPS origin), concentricols (terpenoids derived from the acetate-mevalonates pathway), dalesconol A and B (polyketides), and Helicascolide C (polyketides) [27, 28, 29, 30]. Some of these secondary metabolites are precursors of biologically active medicinal compounds. Chan et al. [31] reported that the genomes of *D. eschscholtzii* harbour several stress adaptation mechanisms for their survival in human hosts. Hence, it would not be surprising for the species to have undergone rapid evolution to select for fitness attributes as well as virulence factors related to pathogenicity in humans. Extensive literature search shows that to the best of our knowledge, this is the first time *D. eschscholtzii* is reported to have been isolated from the leaves of *Musa paradisiaca*.

The good antibacterial activity of *D. eschscholtzii* crude extract against *Bacillus subtilis* (3mm), *Pseudomonas aeruginosa* (5 mm) and *Escherichia coli* (7 mm) respectively could have been as a result of the presence of Pestalotiactone [32]. Talonsi et al [32] reported the isolation of the compound from *Paecilomyces sp.* Strain CAFT156 and it possessed antimicrobial and cytotoxic polyketides.

This result is further supported by Liu et al [33] who reported the isolation of *D. eschscholtzii*, (an endophytic fungus) from medicinal plant *Pogostemon cablin*. Cyclophenol is also known to possess some antimicrobial properties [33]. The metabolites of this fungus possessed antibacterial activity. Gao et al [34] reported that secondary metabolites of *D. eschscholtzii* are rich in Daldionin, a potent antimicrobial agent. When tested against extended spectrum beta lactamase (ESBL) producing *Escherichia coli* and vancomycin resistant *Staphylococcus aureus*, (VRSA) the crude extract showed no activity. Also, the extract showed no antifungal activities.

The extract of the endophytic fungus from *M. paradisiaca* represents a dependable source of bioactive compounds, due to the wide range of compounds with diverse biological properties present in this extract. The HPLC analysis of the extract revealed the presence of pestalotiactone A, and cyclophenol. Upon further analysis of fraction (Bal 4-5-1), the presence of two other compounds aureonitol and palitantin was suggested. The HPLC chromatogram of the fungal extract and its fraction, as well as the UV-spectra and chemical structures of detected compounds are presented in Figures 2 – 4.

Result of extensive literature search shows that this is (to the best of our knowledge) the first time the compound cyclophenol is reported to have been detected and identified in the secondary metabolite of *D. eschscholtzii*, and it's also the first time its isolated from a leave of *Musa paradisiaca*.

The *D. eschscholtzii* crude extract showed a robust free radical scavenging activity (65.9 % inhibition). None of the compound identified has not been previously reported to possess antioxidant activity. The cytotoxic activity of *D. eschscholtzii* crude extract could be attributed to the presence of Pestalotiactone A and maybe aureonitol which is known and proven cytotoxic agent have been reported to be isolated from fungus-bacterium co-cultures [35]. The presence of aureonitol could be assumed to have contributed to the antiviral activity of against HIV-1. Sacramento *et al.* [36] revealed that aureonitol inhibited influenza by targeting conserved residue on the viral hemagglutinin (HA), and that aureonitol inhibited influenza replication at nanomolar concentrations, with a very low cytotoxicity. They stated that the compound presented a very safe range to be used invitro and was approximately 100 times more potent than other fungal derived natural product previously studied against influenza. They indicated that aureonitol's chemical structure may be of interest for further development of anti-influenza drugs [36]. Palitantin is an antifungal and antiprotozoal compound used in biochemical research. Palitantin possess moderate antimycobacterial activity [38, 39].

5. Conclusion

This study represents the first case scenario of *D. eschscholtzii* being isolated from *M. paradisiaca* leaves. This study has shown that endophyte resident in *M. paradisiaca* could synthesize biorelevant compounds that can be useful for antimicrobial, antioxidant, and for antiviral purposes. The analysis of *D. eschscholtzii* extract yielding two known biorelevant compounds (Pestalotiolactone A, and cyclophenol) which are being reported for the first time in *D. eschscholtzii*; and with the suggestive presence of two other biorelevant compounds (aureonitol and palitantin) in the methanol fractions of *D. eschscholtzii* extract upon HPLC-DAD analysis. The extract proved to be relevant in the development of drug molecules to be used for combating drug resistance. They could also be subjected to further bioactivity testing to determine how best they can be applied for the benefit of man. They could also be chemically modified to achieve much more effective compounds.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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