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Endophytic Fungal Biodiversity and Antimicrobial Bioprospecting of *Penicillium citrinum* KIB4 from *Khaya ivorensis Bark*

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ABSTRACT

Background: The emergence of pathogens that are resistant to antibiotics has raised serious concerns about public health, forcing the hunt for alternative antibacterial substances. Endophytes, particularly those from unscreened plants, may offer new sources of antibacterial substances. Hence, this research aimed to isolate antibiotic-producing fungi from *Khaya ivorensis* bark, a medicinal plant widely used in Nigeria.
Methods: The fungal endophytes were isolated following conventional methods using potato dextrose agar (PDA). They were characterized by morpho-microscopical and sequencing of the internal transcribed spacer (ITS) of the fungal rDNA. The phylogenetic tree was drawn following the Neighbour-joining method and the tree was annotated using the Interactive Tree of Life (iTOL) version 6.0. The agar plug assay method was used to select putative antibiotic-producing isolate for further research. The antimicrobial activity of the fungal fermentation product was assessed by agar well diffusion while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were estimated using the solid agar dilution technique.
Results: A total of 12 endophytic fungi were isolated. They were noted to be mostly *Aspergillus* sp. (11 isolates) while only one isolate represented *Penicillium* species. Only isolate KIB4 showed good inhibitory activity against several bacterial pathogens tested in the preliminary study within 10 mm to 12 mm inhibition zone diameter (IZD) range. Molecular identification proved that strain KIB4 fell into the genus *Penicillium* and shared the highest ITS gene sequence similarity with *Penicillium citrinum* D112 (98.83%), and *Penicillium* sp. TWJL1743 (98.65%). The fermentation extract of *Penicillium citrinium* KIB4 showed specific inhibition against *Escherichia coli* ML475, *Staphylococcus aureus* LN001, *Klebsiella pneumoniae* ML602, *Bacillus subtilis* ML225, and *Proteus* sp. UR979 with an IZD range of 11mm to 23mm.
Conclusion: We reported for the first time, the antimicrobial efficacy of fermentation extract from endophytic fungi isolated from *Khaya ivorensis* against several bacterial pathogens. Further fractionation of the crude extract is needed to isolate specific antimicrobial compounds.

1. Introduction

Endophytes, a significant part of the fungal diversity, spend a portion of their life cycle in plant tissues without obviously harming the kind of plant they are living on[1]. Endophytes have a wide range of hosts and are numerous species[2]. Endophytes are recognized to inhabit specific biological niches, are capable of surviving in harsh and hostile environments, and are known to create secondary metabolites like those of the host. The secondary metabolites produced by endophytic fungi, in exchange for the space and nutrition provided by plants, have been shown to increase plant growth, increase disease and pest resistance in host plants, improve plant sensitivity to environmental stress, and recycle nutrients[3]. The study of

endophytes is particularly helpful in the process of finding new drugs because nature has an infinite supply of unique bioactive compounds[4]. Natural products (NPs) are the name given to these bioactive compounds. Natural products come from living things including plants, animals, and microorganisms as metabolites or by-products[5]. Since ancient times, natural ingredients have been integral to the development of new medicines. Natural products have been employed as a source of medication because they are affordable and readily available, particularly in underdeveloped nations[6]. They have a variety of bioactivities, including antibiotics, anticancer, antimalarial, anti-inflammatory, and analgesics, and are chemically varied. Plant organisms, particularly fungal endophytes, have produced thousands of novel secondary metabolites with a wide variety of very complex chemical entities, demonstrating that they are a rich source of new structural classes of secondary metabolites. These endophytes secrete bioactive secondary metabolites to defend their hosts against pathogens and harmful circumstances[7]. Marine invertebrates, plants, and soil microbes are anticipated to be some of the most promising resources for the discovery of novel medications[8].

Discovering novel compounds for human therapies, particularly antibiotics, depends on biodiversity studies of endophytic bacteria for pharmacological and biotechnological applications[9]. The development of novel compounds for pharmaceutical usage in drug research, industrial use, and agricultural uses has a tremendous potential to be made possible by microorganisms[10]. Microorganisms are much more diverse but have received much less research than other natural sources like plants[11]. Only around 1% of bacteria and 5% of fungi have been described, according to studies based on estimates of microbial populations. The benefits to human well-being of the remaining ones are still unknown[12]. Additionally, natural compounds or their derivatives make up more than 60% of anticancer and 70% of antibacterial medications currently being used in clinical settings. Today, endophytes are viewed as a crucial part of biodiversity. They might also be a large source of untapped genetic variety. These endophytes have numerous practical uses, such as a source of novel secondary metabolites for pharmaceuticals and other industrial uses, as well as a possible biocontrol agent. Endophytes can defend their host from disease, stress, and insects, among other things[13]. Endophyte colonization and spread have the potential to be major biological agents, particularly in drug discovery[14]. As it is probable that their therapeutic properties are a result of the metabolites produced by their endophytic population, plants employed in traditional medicine have played a significant role in the quest for new bioactive strains of endophytic fungi[15]. Endophytes from different plants were studied widely. However, fungal endophytes from *Khaya ivorensis* have not been documented. Hence, in this study, an attempt was made to unveil the diversity of fungal endophytes inhabiting this medicinal plant and evaluate their antibiotic-biosynthetic potential. This is therefore a step toward the development of a new antibacterial agent that would contain the global menace of antimicrobial resistance, hence promoting sustainable development goals, especially as regards global good health and well-being.

2. Materials and methods

2.1 Materials

Potato dextrose agar (PDA, Himedia), Mueller Hinton agar (MHA, Himedia), Nutrient agar (NA, Himedia), Quick-DNA™ Fungal/Bacterial kit (Zymo Research; Catalogue No: D6005), OneTaq Quick-Load™ 2X master mix (Catalogue No: M0486), ITS1 and ITS4 primers (Inqaba Biotech., South Africa), BrilliantDye™ Terminator cycle Sequencing kit (V3.1, BRD3-100/1000), DNA sequencing clean-up kit™ (Zymo Research, ZR-96; Catalogue No: D4050), ABI 3500XL Genetic Analyzer (Applied Biosystems; ThermoFisher Scientific), sterile swab stick, 70% ethyl alcohol, 4% sodium hypochlorite, sterile water, streptomycin antibiotic powder, gentamycin injection, sterile knife, forceps, agar plates, Erlenmeyer flask, ethyl acetate (Sigma Aldrich, Germany), cork-borer, dimethyl sulphoxide (DMSO; Sigma Aldrich, Germany), rotary evaporator, cheesecloth.

2.2 Collection and authentication of plant sample

Healthy bark of *Khaya ivorensis* that were free from insect, infections and mechanical damages were bought from Olosha herbal market (6.5290°N and 3.3537°E) Mushin, Lagos state, South-Western Nigeria in March, 2023. They were packed in a ziplock bag and transported immediately to the laboratory for analysis. The sample was authenticated and archived at the herbarium of the Department of Botany, University of Lagos, Akoka with the voucher number LUH 9076.

2.3 Source of test bacteria

The bacterial species (*Escherichia coli* ML475, *Staphyolococcus aureus* LN001, *Klebsiella pneumoniae* ML602, *Bacillus cereus* ML225, and *Proteus* sp. UR979)

used in this study were obtained from the Department of Microbiology, University of Lagos Teaching Hospital (LUTH), Lagos, Nigeria. Their identities were further confirmed in the Laboratory using Vitek® 2 system. Stock cultures were maintained on nutrient agar slant at 4 °C until needed.

2.4 Surface sterilization of plant samples for endophytic fungal isolation

The collected bark samples were thoroughly washed with running tap water 3 times and surface sterilized by immersing into 70% ethyl alcohol for 1 minute, treated with 4% sodium hypochlorite solution for 3 min, and again rinsed with 70% ethyl alcohol for 1 minute[16]. They were further rinsed with sterile water 3 times. A sterility test was thereafter conducted by plating 1 mL aliquots of the sterile distilled water used in the final rinse onto the surface of potato dextrose agar (Himedia, India) plates supplemented with streptomycin (100 µg/mL) and the plates were examined for growth after incubation at 25°C for 3-5 days. After sterility confirmation, the sterile bark samples were reduced to tiny bits (1 x 1 cm^2) with the aid of a sterile knife and aseptically inoculated onto sterile PDA pre-supplemented with streptomycin (100 µg/mL) to isolate the endophytic fungi. The inoculated plates were incubated at 25 °C and examined daily for 10 days for the development of fungal colonies growing out from the segments. Based on morphological characteristics, the different fungal mycelia growing out of the sample plates were selected, sub-cultured and the pure endophytic fungi maintained on PDA plates[17,18].

2.5 Selection of putative endophytic fungi harboring antibacterial biosynthetic potential

The choice of putative endophytic fungi isolates was selected based on the result obtained from agar plug assay[16]. Each fungal endophyte was grown on the PDA plate for ten days to allow the production of antibiotic peptides if any. The bacterial lawn was prepared by swabbing the MacFarland concentration of each pathogen on a Mueller Hinton agar. A 6 mm cork borer was now used to bore the fungal endophyte and aseptically placed the same onto the already swabbed plates containing different pathogenic bacteria. The setup was allowed to be incubated for 4 hours at 4 °C to enable adequate diffusion of the antimicrobial compounds into the medium if any before the setup was finally incubated at 37 °C for 24 hours. Any zone of inhibition around the endophytic agar plug was measured using a calibrated ruler.

2.6 Extraction of DNA and amplification of PCR of the putative fungal isolate

The extraction of fungal genomic DNA, amplification of PCR, and Sanger sequencing of the amplicon were performed at Inqaba Biotec® Genomic Company Ibadan, Nigeria. Genomic DNA was extracted from the sample using the Quick-DNA™ Fungal/Bacterial kit (Zymo Research). The ITS target region was amplified using OneTaq Quick-Load™ 2X master mix (Catalogue No: M0486) with the universal primers, ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3'). The PCR products were run on a gel and were later cleaned up by EXOSAP method[19]. The extracted fragments were sequenced in the forward and reverse direction and purified using DNA Sequencing Clean up kit™. The purified fragments were analyzed by using ABI Genetic analyzer and the obtained results were finally blasted on the NCBI database[19,20].

2.7 Sequence and phylogenetic analysis

The resulting DNA sequences were aligned using MUSCLE software embedded in MEGA X. They were manually trimmed and edited to obtain complete sequence. Homology searches were carried out using BLASTn program against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic tree was constructed from the evolutionary distance using the Neighbour-joining method performed with MEGA X. The tree was annotated using Interactive Tree of Life (iTOL) software version 6.0 (https://itol.embl.de/). The confidence level of each clade was estimated using bootstrap analysis (1000 replicates).

2.8 Fermentation and extraction of putative endophytic fungal metabolites

The method described by Sathiyaseelan et al. was followed[21]. Potato dextrose broth (PDB) was prepared according to specification and autoclaved. Then, 300 mL was aseptically measured and dispensed into 1L of Erlenmeyer flasks each for the cultivation of the fungal isolates. The medium was later inoculated with six-disc plugs (6 mm in diameter) of the mycelia from already identified putative endophytic fungi isolate grown on PDA for 7 days. All the flasks were plugged with sterile cotton wools and incubated at room temperature 25 °C for 10 days with periodic manual shaking. The fermentation broth of each endophyte was filtered through cheesecloth to separate the mycelia biomass. The filtrate was then extracted exhaustively three times ethyl acetate (1:1) in a

separating funnel by vigorous mixing for 1 hour. The organic phase (Ethyl acetate) containing the fungal metabolites was separated and concentrated using a rotary evaporator to obtain the crude metabolite extract for antimicrobial assay.

2.8.1 Bioassay of crude metabolite extract of endophytic fungal cultures

The antibacterial activity of the crude extract was determined by agar well diffusion method using Mueller Hinton agar medium. All the overnight bacterial cultures were adjusted to 0.5 McFarland standard[22]. Tested microbes were inoculated into Mueller Hinton agar plates using a sterile cotton swab. About a 6 mm size well was made using a sterile cork borer and an amount of 100 µL of crude endophytic fungal metabolite extract dilution (200 mg/mL) was added into each well on different plates. Exactly 100 µL of gentamicin (2 mg/mL) was used as positive control while 10% DMSO was used as negative control. The Petri dishes were left on the bench for about 4 hours to allow the extract to diffuse properly into the agar. They were then incubated at 37 °C for 24 hours and zones of inhibition were observed and measured using a transparent ruler in millimeters. The procedure was carried out in triplicate.

2.8.2 Determination of minimum inhibitory concentration (MIC)

The solid dilution method using double-strength Mueller-Hinton agar was used to determine the putative extract's minimum inhibitory concentration (MIC). A stock solution of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 3.125 mg/mL of each extract was made by pipetting the same volume of 5 mL of sterile diluent (10% DMSO) into a sterile petri dish and aseptically pouring 5 mL from each stock solution. The petri dishes were then inoculated with 100 µL of each of the standardized test bacterial isolates. Additionally, as the experimental control, Muller Hinton agar twice its normal strength in a petri dish was infected with each of the standardized test bacterial isolates. All the petri dish cultures, together with the experimental controls, were incubated for 24 hours at 37 °C, and the growth of each was observed. The lowest concentration at which bacterial growth was inhibited was the minimum inhibitory concentration (MIC) of the extract[23].

2.8.3 Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentrations (MBC) experiment was carried out by inoculating fresh nutrient agar plates using one loop-full of culture from each cultured plate that showed no growth, including the MIC plates. At 37 °C, the plates were incubated for 24 hours. The lowest dose of extract that killed the bacteria on the solid medium was used to compute the MBC value after the incubation period[23].

2.9 Statistical Analysis

Calculation of means and standard deviations was done using Microsoft Excel Office 2007 version. Results of the antimicrobial activity were written as X ± Standard deviation, where X is the average number.

2.9.1 Data availability

Sequence data are available at NCBI GenBank (https://www.ncbi.nlm.nih.gov/). The accession number assigned to *Penicillium citrinium* KIB4 is OR469270.

3. Result

3.1 Isolation and microscopy of endophytic fungi

A total of 12 endophytic fungal isolates was isolated from the bark of *Khaya ivorensis*. Their morphological characteristics on the potato dextrose agar (PDA) were consistent with that of the genus *Aspergillus* while only isolate KIB4 was consistent with the genus *Penicillium* (Figure 1).

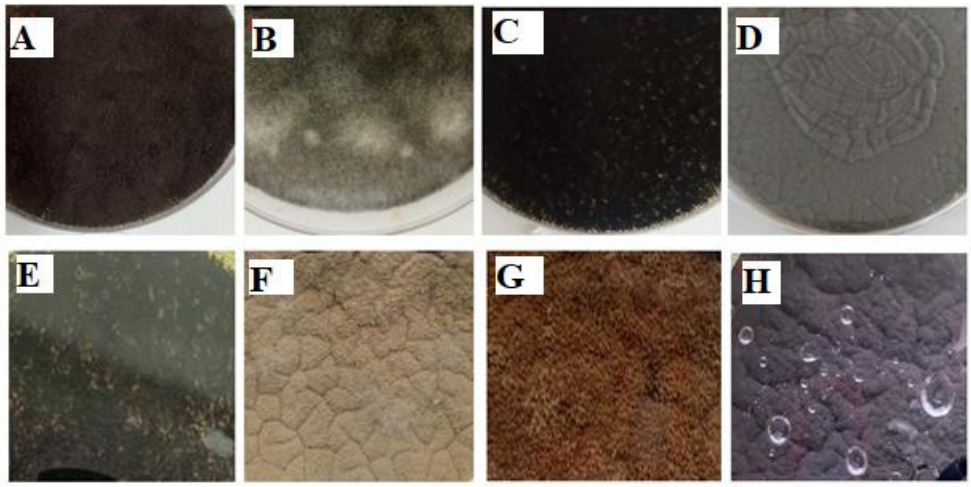

Figure 1: Morphological appearance of diverse endophytic fungi from *K. ivorensis* bark. Letters (A), (B), (C), (D), (E), (F), (G) and (H) represent isolate KIB12, KIB9, KIB6, KIB4, KIB10, KIB11, KIB5 and KIB1 respectively. The isolates were grown on PDA media at 28 °C for 10 days.

The microscopical features of the isolates includes presence of hyphaes, conidia, microconidia, macroconidia, phliades and several spores (Figure 2)

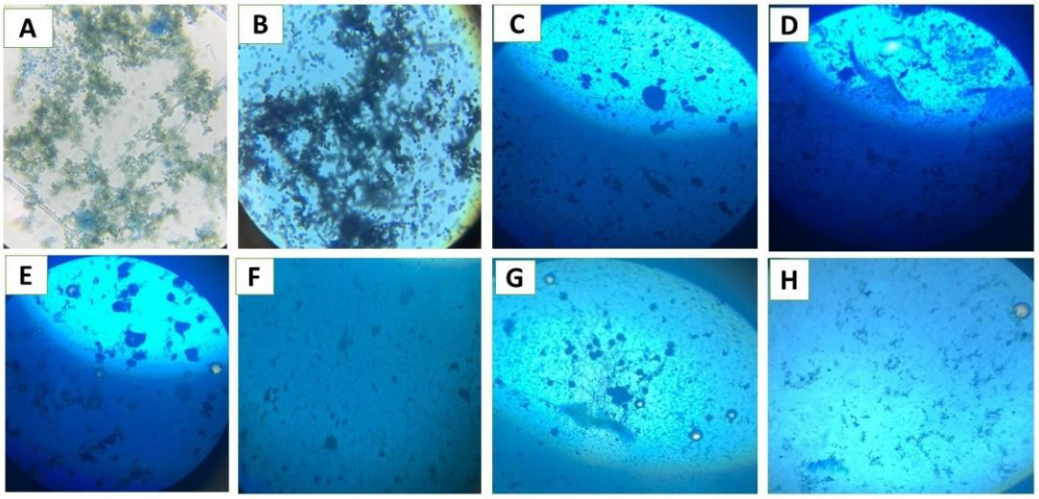

Figure 2: Microscopical representation of different fungal endophytic isolates from *K. ivorensis* bark. Letters (A), (B), (C), (D), (E), (F), (G) and (H) represent isolate KIB12, KIB9, KIB6, KIB4, KIB10, KIB11, KIB5 and KIB1 respectively. Lactophenol cotton blue stain was used to mount fungal samples which were examined at 40X and 100X magnification using OLYMPUS® microscope.

3.2 Selection of putative fungal endophytes by agar plug assay.

The preliminary antimicrobial bioprospecting assay for these endophytic fungi showed that only the isolate represented by KIB4 was able to inhibit all the bacterial pathogens tested (Figure 3). The inhibition zone diameter was within the range of 10 to 13mm against all the tested pathogens.

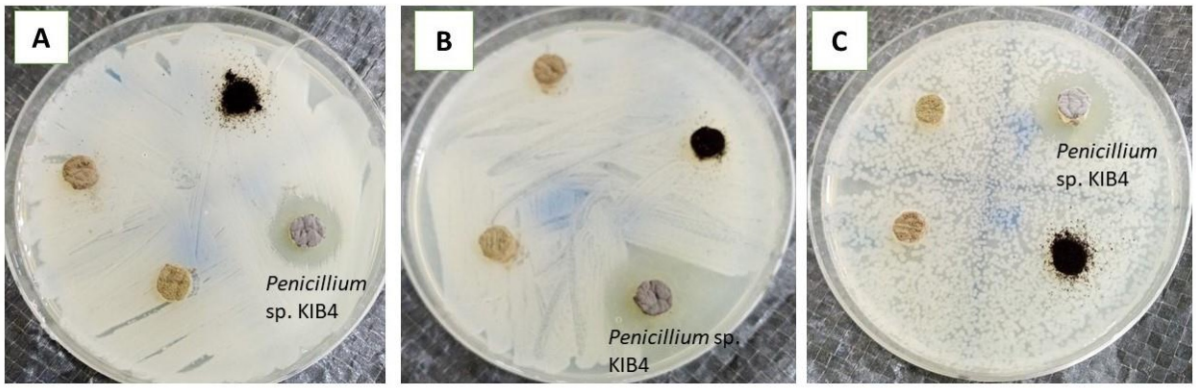

Figure 3: Preliminary antimicrobial screening of fungal endophytes from *K. ivorensis* against pathogenic bacteria. (A), **(B)** and **(C)** Show the antibacterial activity of *Penicillium* sp. KIB4 against *B. subtilis*, *Proteus* sp. and *Staphylococcus aureus* respectively.

The rest eleven isolates did not show any inhibitory activity during the preliminary study. Hence, only the isolate KIB4 was further characterized by sequencing the internal transcribed spacer (ITS) region of the rDNA.

3.3 Morphological features of *Penicillium citrinium* KIB4

The bioprospecting endophytic fungal isolate (KIB4) showed a very light greenish colour on potato dextrose agar (PDA) with a yellow reverse colour (Figure 4).

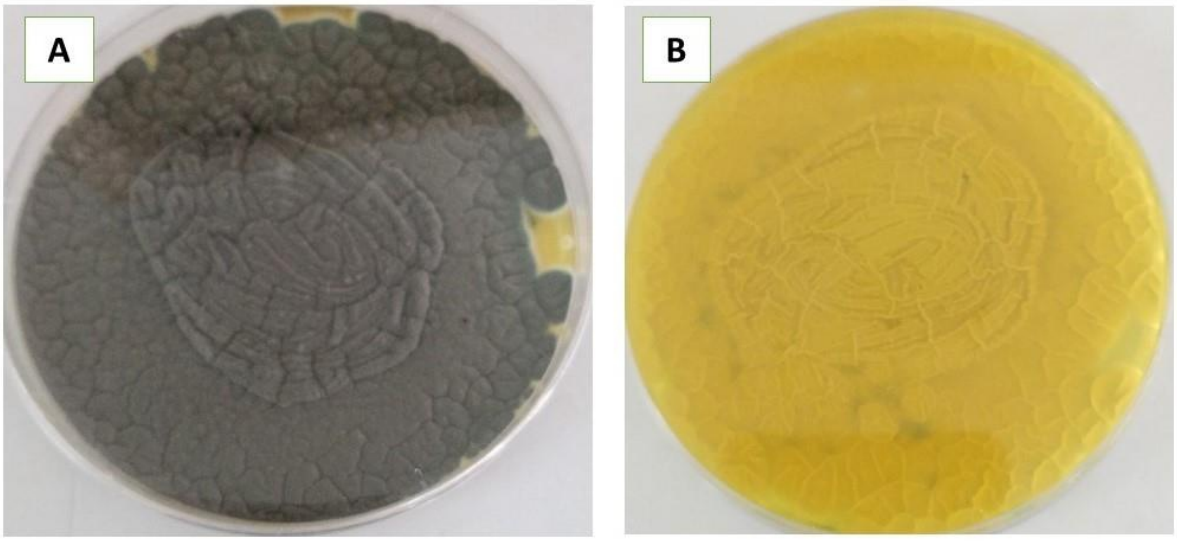

Figure 4: Morphological representation of *Penicillium* sp. KIB4 with antimicrobial bio-prospects. (A) represents the front view of the fungus on PDA plate incubated at 28 °C for 10 days while **(B)** represents the reverse plate of the fungus.

3.4 Phylogenetic analysis

The phylogenetic analysis showed that our isolate had a close ancestral relationship with *Penicillium* sp. TW_JL17_43, *Penicillium citrinum* SC54B06 (MW113595.1) and *Penicillium citrinum* GXIMD02062 (OQ632588.1) (Figure 5).

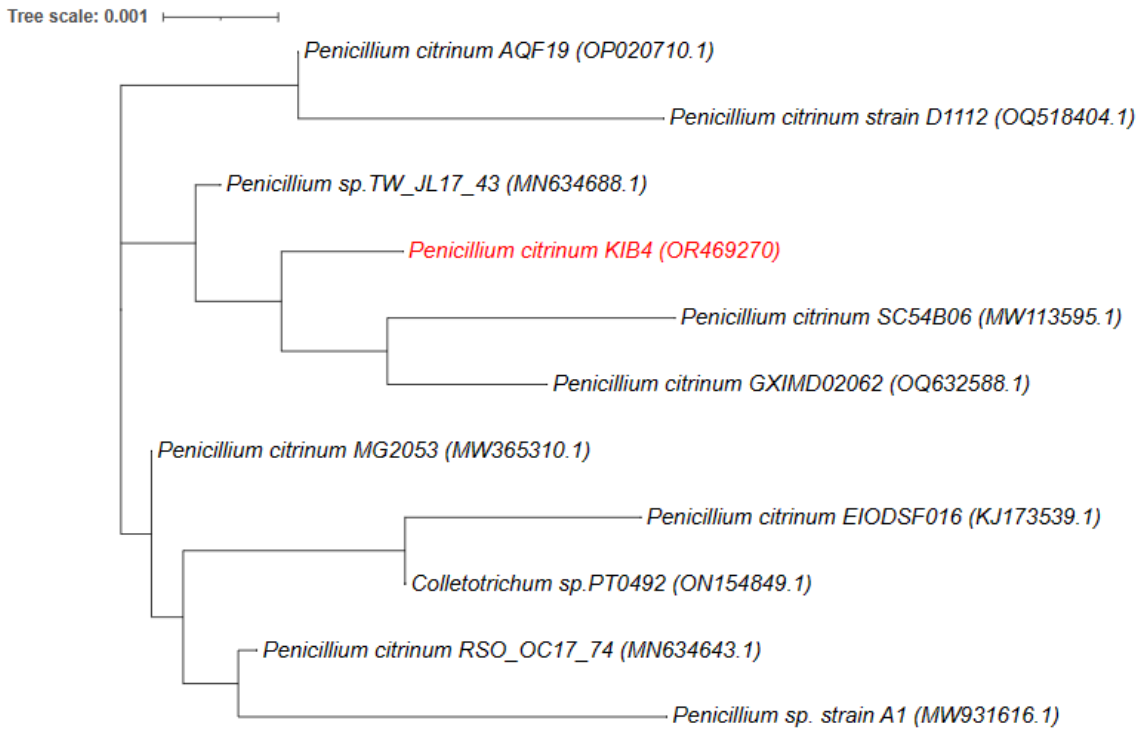

Figure 5: Phylogenetic tree constructed using Neighbour-Joining method for our isolate and other fungi from the NCBI database. Our strain was highlighted in red while the numbers in parenthesis are the organisms' accession numbers. The bar scale depicts 0.001 nucleotide substitutions per site.

3.5 Antibacterial activity of endophytic fungal extract

Antibacterial activity of the endophytic fungi extract showed inhibitory activity against five pathogenic bacteria tested at the range of 11 mm to 23 mm inhibition zone diameters. This is comparable to the range of inhibition zone (14 mm to 24 mm) shown by gentamicin (Table 1, Figure 6)

Table 1: Antibacterial activities of endophytic fungal extract

Samples	Inhibition zone diameters (mm)				
	PR	**S**	**E**	**B**	**K**
KIB4	23±1.3	19±0.5	17±1.2	11±0.3	14±0.8
Gent	24±1.5	21±2.2	18±0.9	22±1.3	14±1.2
10% DMSO	0	0	0	0	0

DMSO stands for dimethyl sulphoxide, GENT – gentamicin, KIB4 – fungal extract of *P. citrinum* KIB4 (Or469270), PR - *Proteus* sp., S - *Staphylococcus aureus*, E - *Escherichia coli*, B - *Bacillus cereus*, K - *Klebsiella pneumoniae*.

Figure 6: Representation of plates for antimicrobial assay by agar well diffusion. Each plate contained a swab of MacFarland concentration of the organism with three 6 mm bored holes, each containing the fungal extract, DMSO and gentamicin. The set up was incubated at 37 °C for 24 hours. DMSO stands for dimethyl sulphoxide, GENT-gentamicin, KIB4 – fungal extract of *P. citrinum* KIB4 (OR469270), PR - *Proteus* sp., S - *Staphylococcus aureus*, B - *Bacillus cereus*, and K - *Klebsiella pneumoniae*.

3.6 Minimal inhibitory and Minimal bactericidal concentrations of the fungal extract

The fermentation product of *Penicillium citrinum* KIB4 showed minimum inhibitory and minimal bactericidal concentrations in the range of 0.32 to 10.40 mg/mL and 0.32 to 20.98 mg/mL respectively. *B. subtilis* and *Proteus* species showed greater resistance as a higher dose of the extract was required to achieve minimal inhibition (Table 2).

Table 2: Minimal inhibitory and minimal bactericidal concentrations of extract putative fungi isolate KIB4

Organisms	*S. aureus*	*E. coli*	*B. subtilis*	*Proteus* sp.	*Klebsiella* sp.
MIC (mg/mL)	0.32	1.28	10.40	10.40	1.72
MBC (mg/mL)	0.32	1.72	20.98	20.98	10.24

4. Discussion

For years, fungal endophytes have served as repositories of bioactive secondary metabolites that form the backbone of many existing drugs. With the global rise in infections associated with antimicrobial resistance, in addition to the growing burden of non-communicable diseases, such as cancer, diabetes, and cardiovascular ailments, the demand for new drugs that can provide an improved therapeutic outcome has become the utmost priority. The exploration of microbes from understudied and specialized niches like medicinal plants is one of the promising ways of discovering promising lead molecules for drug discovery. In recent years, a special class of plant-associated fungi, commonly called fungal endophytes, have emerged as an important source of bioactive compounds with unique chemistry and interesting biological activities.

This study isolated twelve fungal endophytes from the bark of *Khaya ivorensis* which is a known medicinal plant in Nigeria. These fungi were identified using fungal atlas which compared their morphological and microscopical similarities. Eleven of the isolates representing 91.67% were identified as *Aspergillus* species while only one representing 8.33% was identified as *Penicillium* species. This report is consistence with that of many authors whose report showed the abundance of *Aspergillus* species in medicinal plant samples when compared to other genera. The research on the antibacterial activity of endophytic fungi from a medicinal plant *X. aethiopia* showed that among the 4 fungal endophytes isolated, 3 which represented 75% of all isolates were *Aspergillus* sp. while 1 which represented 25% was *Penicillium* species[18]. Other authors have also reported the same establishing that more abundance of *Aspergillus* genera of endophytic fungi among the community[24,25,26].

The preliminary antimicrobial assay of the fungal endophytes showed that only the isolate KIB4, molecularly

identified as *Penicillium* species possessed antimicrobial activity against all the tested bacterial pathogens. It was able to inhibit bacterial growth at an observable inhibition zone diameter measuring 10 to 12mm. Hence, only this isolate was further fermented and extracted for further antimicrobial exploitation. Several authors have reported many endophytic fungi with several pharmacological activities. Fungal endophytes such as *Phyllosticta*, *Penicillium*, *Eutypella*, *Purpureocillium*, *Talaromyces*, *Lophiostoma*, *Cladosporium*, *Pestalotiopsis*, *Chaetomium*, *Fusarium*, *Gongronella*, *Scedosporium* and *Pseudallescheria have been documented for their significant antimicrobial possession*[27]. *Seven endophytic fungi belonging to Macrophomina spp., Aspergillus niger, Penicillium sp., Trichoderma sp. have been isolated from A. boonei, E. chlorantha and K. africana. They were reported to possess antimicrobial activity against several human pathogens including E. coli, P. aeruginosa, E. faecalis, S. aureus, S. typhi, C. albican*[28].

The extract of Penicillium sp. KIB4 showed antibacterial activity against all the tested bacterial pathogens. The crude extract's activity was comparable to the antimicrobial activity of standard gentamicin antibiotics. The zone of inhibition diameters (IZDs) showed by the crude extract against Proteus sp, S. aureus, E. coli, B. subtilis, and Klebsiella sp. were 23mm, 19mm, 17mm, 11mm and 14mm respectively. It showed greater activity on Proteus sp while the least activity was observed against B. subtilis. The minimum inhibitory concentration (MIC) of the extract against the tested bacteria ranged between 0.32 to 10.40 mg/mL while the minimum bactericidal concentration (MBC) ranged between 0.32 to 20.98 mg/mL. Our results deviated from the work of Amiri and Tibuhwa who reported that the MIC of their endophytic fungal (Penicillium sp., Aspergillus sp., Lasidioplodia sp., Daldinia sp. and Neopestalotiopsis sp.) extracts ranged between 6.25 to 100 mg/mL against Staphylococcus aureus and Escherichia coli[29]. *This disparity could be attributed to the genetic makeup of both the endophytic fungi isolates and the pathogenic strains tested. The antimicrobial secondary metabolites produced by an endophyte may also vary based on its ability to harbour certain biosynthetic gene clusters*[30]. *Moreover, seasonal and geographical differences could also contribute to the variations in the production of antimicrobial secondary metabolites.*

The molecular phylogenetics of Penicillium citrinium KIB revealed our strain to be divergent and nested within the same clade with Penicillium sp. (TW_JL17_43), *Penicillium citrinum* SC54B06 (MW113595.1) and *Penicillium citrinum* GXIMD02062 (OQ632588.1). The phylogenetic tree showed that *Penicillium* sp. TW_JL17_43 was more closely related to our strain than the other two strains in the same clade. However, none of these closely related strains have been reported to possess antimicrobial activity against bacterial or fungal pathogens. Hence, further genomic characterization of *Penicillium sp. KIB4 would be required to ascertain its novelty.*

5. Conclusion

Endophytic fungi from *Khaya ivorensis* may represent alternative sources of antimicrobial agents. *Penicillium citrinum* KIB4 could produce antibacterial peptides against several bacterial pathogens. Its metabolites could serve as novel antimicrobial agents against several drug-resistant bacteria, hence improving the global healthcare system. Further genome mining and biotechnological exploitations of *Penicillium sp. KIB4* are recommended to enable the development of new drug lead and biological control compounds.

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