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Antibacterial Activity and Phylogeny of Griseofulvin Producing *Aspergillus* and *Penicillium* Species from Kunduchi Mangrove Sediments, Tanzania

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Abstract

Marine fungi are an underrated reservoir of bioactive metabolites for drug discovery. This study focused on fungi isolated from Kunduchi mangrove sediments in Tanzania. In an attempt to investigate the antibacterial activities of marine fungi and uncover their bioactive compounds, ethyl acetate crude extracts of fungal isolates were screened for antibacterial activity against human pathogens (*Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*) followed by compounds identification by Gas Chromatography-Mass Spectrometry (GC/MS). A total of seven fungal isolates were obtained from sediments. Crude extracts from isolate 1 and isolate 2 exhibited significant antibacterial activities with a Minimum Inhibitory Concentration > 3.13 mg/ml against *S. aureus* and MIC > 0.78 mg/ml for both *B. subtilis* and *E. coli.* Furthermore, crude extract analysis by GC/MS detected a substantial amount of griseofulvin from the extracts of both isolates 1 and 2. Using nuclear Large Subunit and Internal Transcribes Spacer molecular markers, griseofulvin-producing fungi were identified as *Penicillium aethiopicum* and *Aspergillus sydowii*. Significantly, this marks the first documented instance of griseofulvin production by *A. sydowii* from the marine environment worldwide. Our research has not only identified a new alternative source of griseofulvin from *A. sydowii* but also underscores the importance of optimizing culture conditions to enhance the production of bioactive metabolites by the identified mangrove-derived fungi. Future studies may prioritize on optimization of culture conditions as it could have significant implications for the pharmaceutical industry and the development of novel antibacterial agents.

Keywords: Antibacterial activity; Griseofulvin; Human pathogens; Mangrove sediment fungi.

Introduction

Marine fungi produce secondary metabolites with drug discovery potential and possess various biological properties including but not limited to antifungal, anticancer and antibacterial (Tarman 2020)**.** Previously reported bioactive metabolites with therapeutic potential from crude extracts of marine fungi include; citrinin isolated from marine sponge-associated fungus *P. steckii,* circumdatin C isolated from *A. ochraceus*, fumiquinazoline D and E isolated from *A. sydowii* and griseofulvin isolated from *P. fellutanum* (Zhang et al. 2012, He et al. 2013, Mayavan and Sangeetha 2016, Yao et al. 2021).

Mangroves are biodiversity hotspots for marine fungi that interconnect terrestrial and marine environments (Shearer et al. 2007, Thatoi et al. 2013). These intertidal zones are the best reservoir of organisms that produce bioactive secondary metabolites (Nicoletti and Vinale 2018). *Aspergillus* and *Penicillium* are the most occurring genera of filamentous fungi from marine habitats, predominantly existing as saprophytes (Jones

et al. 2015, Nicoletti and Trincone 2016). Marine fungi from the two genera (*Aspergillus* and *Penicillium*) produce bioactive compounds with medicinal value (Nigam and Singh 2014).

Griseofulvin is an antifungal antibiotic useful in the treatment of dermatophytic infections in humans and animals which works by blocking fungal mitotic processes. It has also been reported to have other biological activities including; antibacterial, anticancer and antiviral (Petersen et al. 2014, Rathod et al. 2014). Initially, it was isolated from the mycelia of *P. griseofulvum* Dierckx by Oxford et al. (1939). Since then, griseofulvin production has been reported from plants (*Vicia faba*) and microbes in laboratory scale and soil (Crowdy et al. 1956). So far, various fungal species have been reported to produce griseofulvin, mostly belonging to the genus *Penicillium.* For instance; *P. raistrickii, P. fellutanum* and *P. urticae* to mention a few. However other genera that accommodate griseofulvin-producing fungal species include*; Nigrospora*, *Nematospora*, *Aspergillus* and *Xylaria* (Nigam and Singh 2014, Rathod et al. 2014, Mayavan and Sangeetha 2016). Relatively less has been done to explore mangrove fungi from Africa. Earlier studies conducted on mangrove fungi focused on the taxonomy and listing of marine fungi (Myovela et al. 2023). Explorations of marine mangrove fungi in Africa have been conducted in Niger, Kenya, South Africa and Nigeria (see for example Akinduyite and Ariole 2019, Osorio et al. 2021, Aina et al. 2021, Kiti et al. 2022). The scarcity of marine fungi research in Tanzania has necessitated studying marine fungi to uncover their bioactive compounds with therapeutic potential. In the course of searching for secondary metabolites with medicinal value, marine fungi were isolated from mangrove sediments, tested for antibacterial activity and griseofulvin-producing fungal isolates (*A. sydowii* and *P. aethiopicum*) were identified.

Materials and Methods

Study area

Kunduchi mangroves are located around the Western Indian Ocean coastal area at Kunduchi ward situated in Kinondoni district found in Dar-es-Salaam region, Tanzania. Sediment samples were collected at the Ununio coastline stretch where mangroves are daily watered by the ocean to maximize the chance of isolating marine fungi (figure 1). The site was selected because only a few studies have been conducted on mangrove fungi from that area. Google Earth images analyzed year 2014 showed that Kunduchi had 157.3 hectares of mangroves and about 40% were endangered by anthropogenic activities (Mabula et al. 2017). Mangroves found at Ununio-Kunduchi have been lost, and some of them modified due to human activities such as trampling, salt production activities, collection of firewood and construction of estates, hotels and residential houses (Idukunda et al. 2020).

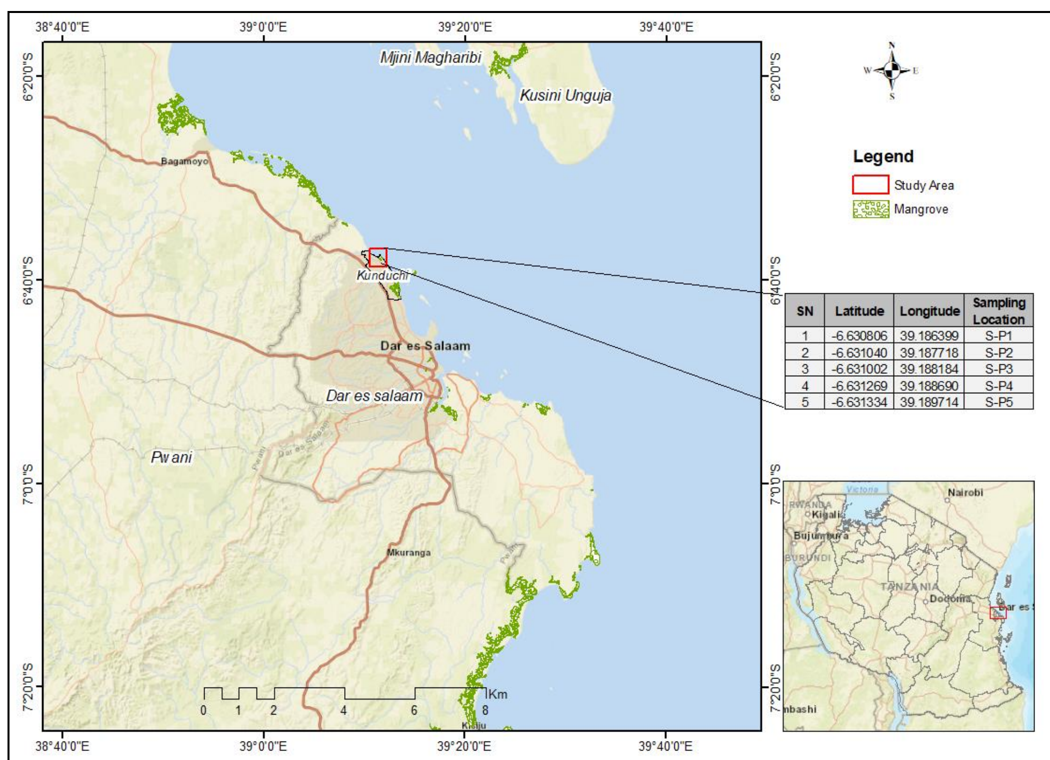

Figure 1: Map locating study area and sampling points where sediment samples were collected in Tanzania

Sample collection and processing

Sampling and sample processing was done as described by Annathurai and Konthoujam (2020) with minor modifications. Briefly, fifteen sediment samples were randomly collected from mangroves of the Ununio coastline stretch (five locations were selected and for each location, a sample was taken in triplicate) Dar es Salaam, Tanzania**.** Sediments were collected 6 cm deep below the pneumatophores using a sterile corer made by cutting off the top of a 50 ml syringe and kept in pre-sterilized plastic bags then transported to the University of Dar-es-salaam Microbiology laboratory for further processing.

Isolation of marine fungi from the sediments

A serial dilution technique was used to isolate marine fungi from sediments. One (1) g of sediments was added in 9 ml of sterile normal saline solution. A ten-fold serial dilution was done for the samples. The 0.1 ml of 10^{-1} to 10^{-4} dilutions were cultured on prepared Potato Dextrose Agar supplemented with chloramphenicol in petri dishes (0.05 g/l for bacteria growth inhibition) using the spread plate method. The PDA media was prepared using 50% filtered natural seawater followed by an adjustment of pH to 5.6 to favor fungal growth. Incubation of petri dishes was done at 28 °C for 96 hours. After the incubation period, fungal isolates were observed then seven different fungal colonies were subcultured on the PDA media to obtain pure cultures (Radu and Kqueen 2001, Jang et al. 2006, Bandaru et al. 2017).

Fungal mass cultivation and extraction of secondary metabolites

Using a sterile cork borer, mycelia agar plugs of six fungal isolates (9 mm) were aseptically cut from fungal pure cultures and deposited in 1000 ml Erlenmeyer flask holding 500 ml Malt extract broth (that was prepared using 50% filtered natural seawater) followed by stationary incubation of the flasks at room temperature (Jang et al. 2006, Mwanga et al. 2019). Extraction of secondary metabolites was carried out in series after the 7th, 14th and 21st day of incubation. Mycelia

mats were removed from fungal cultures by filtration using cotton gauze. An equal volume of ethyl acetate was added in a separating funnel containing filtered broth followed by shaking of the mixture for 10 minutes. The mixture was then left to settle for approximately 5 minutes to allow the separation of the organic layer containing metabolites from the other layer containing the medium which was discarded after collection of the organic layer in a sterile Erlenmeyer flask. Crude extracts were concentrated by removing ethyl acetate (from the organic layer) using a rotary evaporator (RE-501 Henan, CHINA) under reduced pressure at 35 °C (Sandhu et al. 2014, Amiri and Tibuhwa 2021).

Screening for antibacterial activity inoculum preparation

Stock solution preparation

Fungal crude extracts were weighed and dissolved completely in Dimethyl-sulfoxide (10%) making 100 mg/ml stock solution that was stored at 4 ℃ for future use. Screening for the antibacterial activity was done using standard bacteria; *S. aureus* (ATCC 25923), *B. subtilis* (ATCC 6633) and *E. coli* (ATCC 8739) obtained from the University of Dar-es-Salaam (Department of Molecular Biology and Biotechnology). The suspensions of standard bacteria (approximately 1.5 $\times 10^8$ ^{CFU}/ml) were prepared by inoculating fresh bacteria into sterile normal saline solution (5 ml) then, the suspensions were adjusted to match the McFarland standard of 0.5 that was formulated by mixing 0.05 ml of 1.175% $BaCl_2$ and 9.95 ml of 1% H_2SO_4.

The antibacterial activity of fungal extracts against test organisms was determined using the Kirby-Bauer disc diffusion method (Hudzicki 2009). Briefly, 30 µl/disc extract solution (concentration of 50 mg/ml) was impregnated in sterile Whatman paper discs (6 mm diameter), left to dry then placed on the surface of solidified nutrient agar petri dishes seeded with standard test organisms. Test controls were also performed in a similar way using 10% DMSO and chloramphenicol (0.25 mg/ml) as negative and positive control, respectively followed by incubation of petri dishes at 37 ℃ for 24 hours. After the incubation, the diameter for zones of inhibition was measured in millimeters and expressed as Mean ± Standard deviation from the experiment performed in triplicate (Amiri and Tibuhwa 2021).

Determination of minimum inhibitory concentration of fungal crude extracts

Determination of minimum inhibitory concentration (MIC) for the crude extracts of the isolates that exhibited significant antibacterial activity (isolates 1 and 2) was performed using microdilution assay as per Masalu et al. (2020) with minor modifications. Sterile 96 well microplates having 8 rows (A-H) and 12 columns were used. Two-fold serial dilutions were carried out along the columns (from column 1 to column 12) of sterile 96-well microplates yielding eight different concentrations of 100 µl each. The dilutions were performed in triplicate and the concentrations obtained ranged from 100.00 mg/ml to 0.78 mg/ml. Afterward, Muller Hinton Broth inoculated with standard test bacteria with concentration adjusted to match McFarland 0.5 was added in each well halving the concentrations, resulting in a volume of 200 µl in each well. Also, a reference microplate with comparable setup but without the addition of test bacteria was included. Chloramphenicol (0.25 mg/ml) and DMSO (10%) were used as positive and negative controls, respectively. For sterility control, microplate wells with broth only were also included. Microplates were incubated at 37 °C for 24 hours. MIC results were determined using a microplate reader at 630 nm wavelength through a comparison of the average value of three absorbances for each concentration along the columns the of microplate with that of the corresponding concentrations of the reference microplate.

Identification of bioactive compounds from fungal extracts

Crude extracts from fungal isolates that exhibited significant antibacterial activity (isolates 1 and 2) were subjected to Gas Chromatography-Mass Spectrometry analysis as described by Senthilkumar et al. (2014) with modifications. GCMS-QP 2010 (Shimadzu, Japan) with a Restek-5MS column of 0.25 µm film thickness, 30 m

length and 0.25 mm diameter was used. The operating procedures were as follows: 1 µl of the extract which was dissolved in dichloromethane/methanol (1:1) was injected into the chromatograph using split injection mode at the injection temperature of 250 ºC. Helium (carrier gas) had a stream flow rate of 1.2 ml min^{-1}. The oven temperature was programmed to increase to 90 ºC and maintained for 2 minutes (hold time), it was further increased to 280 ºC and maintained for 10 minutes. In the Mass spectrometer, the electron impact mode was at 70 eV and the ion source temperature was 230 ºC. Also, the interface temperature was set at 300 ºC. Compounds were identified using Mass Spectral Library and search software (NIST version 11). Quantification of the compounds from the extracts was done using the Peak integration method whereby ion allowance was 20% target ion and the other five quantitation ions were used in quantitative analysis. The results are reported as percentage compositions derived from the peak area of scanned compounds from the extracts.

Identification of griseofulvin-producing fungi using molecular methods

Extraction of fungal DNA was conducted using the CTAB protocol described by Aboul-Maaty and Oraby (2020) with some modifications. About 100 mg of fungal cultures were placed in sterile Eppendorf tubes then glass beads were added to the tubes followed by the addition of 100 µl of 2× extraction buffers and grinding. To the ground fungi cultures, 500 µl of 2× DNA extraction buffer was added then the samples were placed in a dismembrator for further grinding at 33 revolutions for 1 minute. Samples were then placed in a water bath set at 65 ºC for 1 hour while the mixing was done at 20-minute interval by inversion of the tubes, 20 times each afterward, the mixtures were cooled to attain room temperature followed by the addition of an equal volume of chloroform: Isoamyl alcohol (24:1), mixing by slight inversion and centrifugation at 1300 rpm for 10 minutes. Using the wide bore pipette the upper aqueous phases were carefully transferred to new Eppendorf tubes where an equal volume of chloroform: isoamyl alcohol (24:1) was added again followed by mixing, centrifugation (at 1300 rpm for 10 minutes) and the transfer of 450 µl upper aqueous phases to new Eppendorf tubes. To each volume, 225 µl of 6 M of NaCl was added followed by mixing. Successively 45 µl of 3 M potassium acetate was added and simultaneously mixed with 300 µl of ice-cold 100% isopropyl alcohol. The mixtures were then incubated at -20 ºC for 30 minutes followed by centrifugation at 13000 rpm for 5 minutes. The supernatants were discarded and tubes containing DNA pellets were inverted on tissue paper to completely drain off the supernatants. DNA pellets were then washed with 100 µl of 70% ethanol followed by inversion of the tubes and centrifugation at 13000 rpm for 5 minutes. 70% alcohol was discarded from the tubes, the tubes were then inverted on filter paper to dry at room temperature for 15 minutes. DNA pellets were then re-suspended in 30 µl 1×TE buffer and stored at -20 ºC till further use. DNA concentrations and purity were ascertained by spectrophotometric methods using a Nanodrop spectrophotometer (Nanodrop One, Thermo Scientific, USA) by considering the ratio of absorbance at 260 nm and 280 nm (260/280 nm).

To amplify fungal rDNA internal transcribed spacer (ITS) and Large Subunit (LSU) regions, Polymerase Chain Reactions (PCR) were carried out using primers set ITS1/ITS4 and LROR/LR7 for ITS and LSU respectively. The PCR conditions for ITS included: initial denaturation at 95 °C for 2 min, 35 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec, elongation at 72 °C for 30 sec and final extension at 72 °C for 10 min (White et al. 1990). Also, PCR conditions of the rDNA large subunit (LSU) were: initial denaturation at 95 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, elongation at 72 °C for 45 sec and final extension at 72 °C for 5 min (Hussein et al. 2018).

PCR amplicons were analyzed using 1.5% agarose gel submerged in 1×TBE

(Tris/Borate/EDTA) buffer in gel electrophoresis tanks (Chin et al. 2021). The PCR products from ITS and LSU regions were submitted to Inqaba Bioteq East Africa Limited for purification and Sanger sequencing. Sequences obtained were compared to those sequences present on the NCBI website for the identification of fungal species through BLAST search after the quality control process in Genious v.9.1.2 software.

Phylogenetic analysis

Fungal LSU/ITS sequences were used as query sequences to search for similar sequences from the NCBI database employing the BLASTn program. Thirty-one sequences were selected for the construction of the phylogenetic trees based on high similarity to the query sequences. Sequences were aligned in AliView version 1.28 software, and gaps were considered as missing data followed by phylogenetic tree construction using two methods which are Bayesian Inference and Maximum Likelihood using MrBayes version 3.2.7a and RAxML version 8.2.10 software respectively (Bin et al. 2014). Two outgroups; *Caliciposis beckhausii* and *Caliciposis valentina* were selected from the Coryneliaceae family. For Bayesian analysis, the optimal model of nucleotide substitution was chosen according the to Akaike Information Criterion in MrModeltest v2.3 (Nylander 2004). Two runs of four Monte Carlo Markov Chains were carried out from ten million generations. Trees were sampled every 100 generations and 25% were discarded as a burn-in. Maximum likelihood analyses were performed using the GTR+G+I model (Stamatakis 2014). The branch support was obtained by maximum likelihood bootstrapping of 1000 replicates (Hillis and Bull 1993).

Results

Antibacterial activity of ethyl acetate fungal crude extracts

A total of seven fungal isolates were obtained from mangrove sediments. The presence of a clear zone around discs that contained extracts, was considered as an indicator of antibacterial activity. The results of the zone of inhibition were expressed as mean ± standard deviation which revealed that ethyl acetate crude extracts of six fungal isolates exhibited antibacterial activity against human pathogens which are; *B. subtilis, S. aureus* and *E. coli*. The highest antibacterial activity of the fungal extracts was demonstrated by ethyl acetate crude extracts of isolates 1 and 2. Furthermore, crude extracts of fungal isolate 1 exhibited slightly higher antibacterial activity than crude extracts from isolate 2 (Table 1). The crude extracts from the two isolates were most effective in growth inhibition of *B. subtilis*, followed by *E. coli* lastly, *S. aureus*.

Table 1: Antibacterial activities of ethyl acetate crude extracts of mangrove sediment fungi against medically important pathogens

Fungi ID	Zone of inhibition (mm)		
	Test microorganisms		
	B. subtilis	*E. coli*	*S. aureus*
Isolate 1	13.70 ± 0.58	11.00 ± 0.00	10.30 ± 0.58
Isolate 2	12.00 ± 0.00	10.70 ± 0.58	10.00 ± 0.00
Positive control	25.70 ± 1.20	27.70 ± 1.20	24.00 ± 2.00
Negative control	-	-	-

Positive control = chloramphenicol (0.25 mg/ml), Negative control = DMSO 10%. The results presented in the table are for the isolates that demonstrated the highest antibacterial activity.

Minimum inhibitory concentration (MIC) of fungal crude extracts

Minimum inhibitory concentrations of ethyl acetate crude extracts from marine mangrove sediment fungi which exhibited significant antibacterial activity against test microorganisms are presented in Table 2. Crude extracts from the two isolates (Isolate 1 and 2) exhibited similar antibacterial activity with MIC values > 3.13 mg/ml against *S. aureus* and MIC > 0.78 mg/ml against both *B. subtilis* and *E. coli.*

Table 2: Minimum inhibitory concentrations (mg/ml) of ethyl acetate crude extracts of marine mangrove sediment fungi against medically important pathogens

Fungi isolate	MIC (mg/ml) Test microorganisms		
	S. aureus	*B. subtilis*	*E. coli*
Isolate 1	> 3.13	> 0.78	> 0.78
Isolate 2	> 3.13	> 0.78	> 0.78

Detection of griseofulvin from crude extracts of mangrove sediment fungi

GC-MS analyses of fungal crude extracts revealed a total of 7 compounds with diverse biological activities griseofulvin being the major compound from both fungal isolates. Griseofulvin peak was detected at m/z 138.1 and it was found to contain the same molecular weight (352 Da) but eluted at different retention times that is; 21.602 and 20.534 minutes for isolate 1 and 2 respectively. Peak sizes for griseofulvin detected in the crude extract of fungal isolates 1 and 2 look different suggesting variation in the amount of the compound (Figures 2 and 3). Other compounds detected include; dechlorogriseofulvin and hexadecene from the crude extract of isolate 1 also, hexadecane, 1-hexadecanol and hexadecanoic acid from the crude extract of isolate 2.

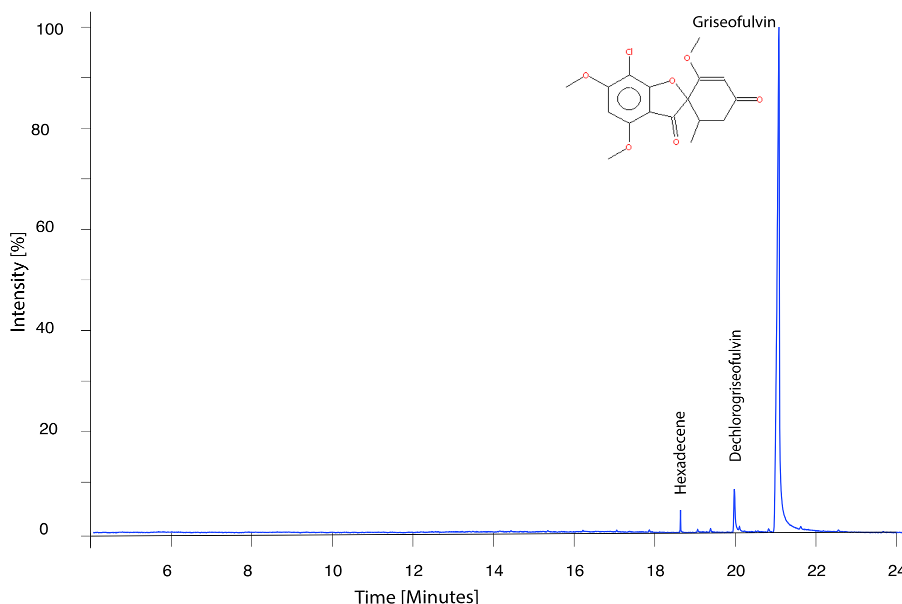

Figure 2: GC-MS Analysis of the ethyl acetate crude extracts of fungal isolate 1 from Kunduchi Mangrove Sediments, Tanzania

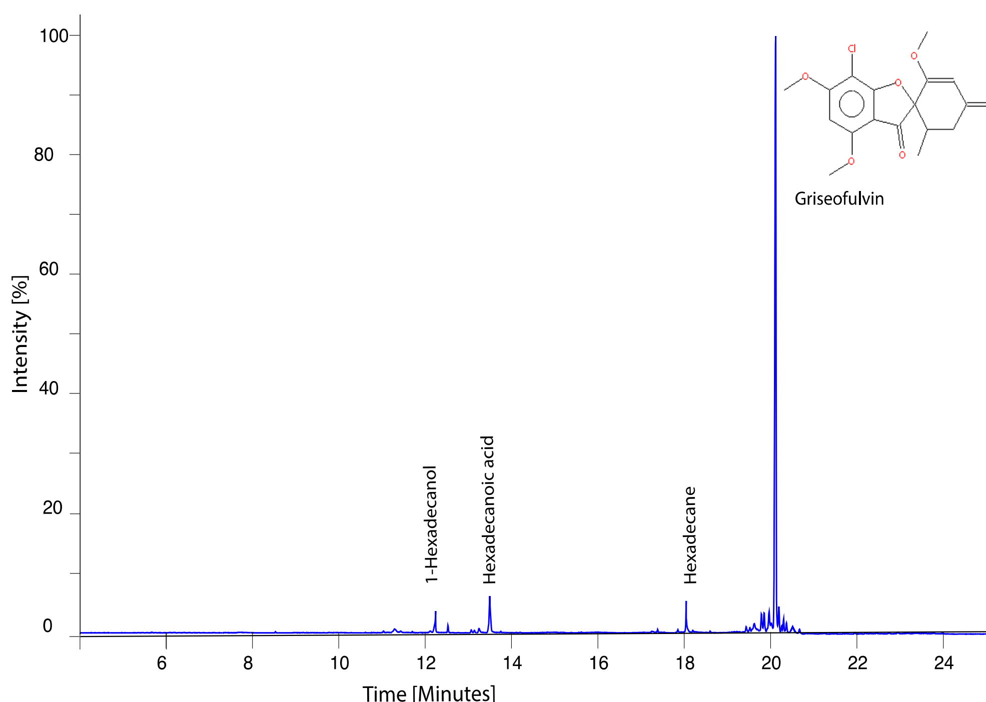

Figure 3: GC-MS analysis of the ethyl acetate crude extract of fungal isolate 2 from Kunduchi Mangrove Sediments, Tanzania

Molecular identification and phylogeny of mangrove sediment fungi

Based on molecular analysis, the two griseofulvin fungal isolates were identified as *P. aethiopicum* (isolate 1) and *A. sydowii* (isolate 2) (Table 3).

Table 3: Fungal species identification through comparison of nrLSU and ITS sequences of griseofulvin producing fungal isolates using the Basic Local Alignment Search Tool with sequences accessible in NCBI GenBank

Fungal isolate ID	Specie name	Accession number		% Identity		Reference
		LSU	ITS	LSU	ITS	
1	*P. aethiopicum*	U15471	AY373896	100%	100%	(Peterson 1997, Haugland et al. 2004)
	P. aethiopicum	**OQ001244**	**OQ001168**			
2	*A. sydowii*	KJ524908	MT582755	100%	100%	(Hallegraeff et al. 2014, direct submission)
	A. sydowii	**OQ001250**	**OQ001174**			

Bolded details are for griseofulvin producing fungal isolates from this study and the unbolded details are for the fungal isolates best match (closest strain) from the GenBank.

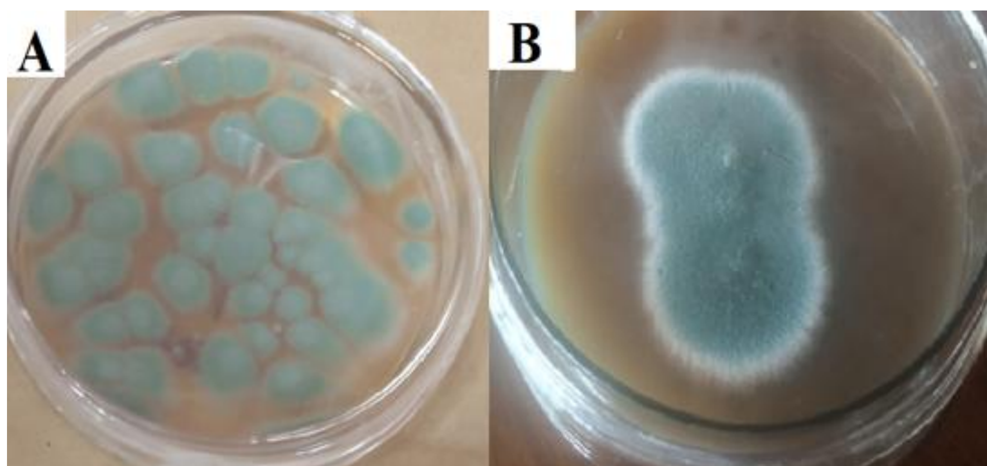

Figure 4: Morphological appearance of griseofulvin-producing fungi isolated from mangrove sediments on potato dextrose agar. A and B show the front view of fungal isolates 1 and 2, respectively

Figure 5: Phylogeny of griseofulvin-producing fungi isolated from mangrove sediments inferred from Bayesian and Maximum likelihood analyses of nrLSU and ITS concatenated datasets. The two values correspond to posterior probabilities and Maximum likelihood bootstraps proportions, respectively

Discussion

Only fungal isolates that exhibited significant antibacterial activity were identified in this research. The identified fungal isolates namely; *P. aethiopicum* (isolate 1) and *A. sydowii* (isolate 2) belong to phylum Ascomycota. Likewise, previous studies have disclosed the prevalence of Ascomycetes in marine environments (Sarma and Hyde 2001, Thatoi et al. 2012). Both

isolates are classified under the class Eurotiomycetes, Aspergillaceae family and order Eurotiales (Houbraken and Samson 2011). The two isolates belong to two different genera which are *Aspergillus* and *Penicillium*. These findings align with previous studies conducted from hypersaline environments which report *Penicillium* and *Aspergillus* as fungal genera with species which have been repeatedly isolated from marine ecosystems (Chameck et al. 2019). The concatenated phylogenetic tree was strongly supported with the branch node values being higher than the cut-off points at PP ≥ 0.95 and Mlb ≥ 70% (figure 5). Marine fungi have gained recognition as potential sources of bioactive secondary metabolites with diverse properties including; antimicrobial, antifungal, antioxidant, anti-fouling and anti-inflammatory activity (Devi and Jayaseelan 2020). These bioactive metabolites play a crucial role in enhancing the survival of marine fungi in their extreme environment (Jin Xu 2015, Reich and Labes 2017).

A significant discovery from this study is the identification of griseofulvin as a major compound detected in the crude extracts of *A. sydowii*. Griseofulvin was also detected in the crude extracts of *P. aethiopicum.* Griseofulvin is known for its antifungal properties and is commonly used in the treatment of fungal diseases affecting humans, animals and plants. It is active in vitro against dermatophytes species which belong to three genera namely; *Epidermophyton*, *Trichophyton* and *Microsporum* (Aggarwal and Goindi 2012). It works by inhibiting fungal cell mitosis (Mayavan and Sangeetha 2016). Previous studies have reported the production of griseofulvin by microbes and plants. Rathod et al. (2014) reported the presence of griseofulvin from the crude extracts of *Nigrospora oryzae* which exhibited antimicrobial activity against both human and plant pathogenic bacteria (*E. coli* and *S. aureus*) and fungi (*Trichophyton mentagrophytes*, *Microsporum canis* and *Fusarium oxysporum*) at MIC less than 1.5 mg/ml for all test organisms. This aligns well with the findings of this study where the crude extract of *A. sydowii* inhibited the growth of *E. coli* at MIC > 0.78 mg/ml. On the contrary, the growth of *S. aureus* was inhibited at MIC > 3.13 mg/ml, a higher value compared to what was reported by Rathod et al. (2014). Results for antibacterial testing imply that crude extracts from both *A. sydowii* and *P. aethiopicum* possess broad-spectrum activity, inhibiting the growth of bacteria of medical importance thus making the two fungi beneficial from a therapeutic standpoint. The analysis of the crude extracts from both *A. sydowii* and *P. aethiopicum* revealed the presence of additional compounds although in trivial amounts hence the implication that, the antibacterial activity of the two fungal species may be substantially contributed by the presence of griseofulvin from the crude extracts**.** Griseofulvin analog, dechlorogriseofulvin was also identified as one of the components of *P. aethiopicum* crude extracts. Dechlorogriseofulvin has been previously reported to have antifungal activity (Shang et al. 2012). Both griseofulvin and dechlorogriseofulvin have been previously isolated from *P. aethiopicum* (Frisvard and Filtenborg 1989). Hexadecene detected in the crude extracts of *P. aethiopicum* has been reported to possess antimicrobial activity (Nguyen and Cao 2022). Palmitic acid (hexadecanoic acid), hexadecane and 1-hexadecanol detected from the crude extracts of *A. sydowii* have been reported to have antimicrobial and antioxidant activities (Chandrasekaran et al. 2005, Mishra and Sree 2007, Fatima et al. 2017, Hwang et al. 2020).

Conclusion

Griseofulvin has been used as an antifungal agent to treat mycotic infections for over forty years. In this study, we have identified griseofulvin in the crude extracts of both *P. aethiopicum* and *A. sydowii* at remarkably high concentrations compared to other compounds. This study accentuates the potential of utilizing these fungi as a promising source of griseofulvin, an antifungal antibiotic that is a promising antibacterial agent against human pathogens.

Further research is recommended on the purification of the components present in the extracts and testing for their antibacterial activity. This analysis will help to determine whether the other compounds within the crude extracts, aside from griseofulvin, possess significant antibacterial properties potentially resulting in a synergistic effect when combined. Furthermore, an investigation into the optimization of culture conditions for both *A. sydowii* and *P. aethiopicum* is warranted. Optimization of culture conditions will maximize the production of griseofulvin and other potential bioactive metabolites from *A. sydowii* and *P. aethiopicum* consequently expanding our understanding into their pharmaceutical applications.

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Declaration of Interest: The authors declare no conflict of interest.

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