Molecular Characterization and Antibacterial Potential of Endophytic Fungal isolates from selected Mangroves along the Coastline of Kenya

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Abstract

The increasing emergence and re-emergence of resistant pathogenic microbes causes a health threat to the human population. This study aimed to characterize mangrove endophytic fungi and evaluate their antibacterial activity. *Heritiera littoralis*, *Rhizophora mucronata, Bruguiera gymnorrhiza*, *Avicennia marina* and *Xylocarpus granatum* species were collected from Tudor Creek, Mida Creek and Gazi Bay. A total of 30 fungal isolates were subjected to molecular identification based on analysis of their ITS gene region. The isolates in the inferred phylogenetic trees were affiliated with the genus *Aspergillus*. Ethyl acetate and butanol crude extracts of 29 fungal isolates and eight mycelia samples were screened for antibacterial activity against *Staphylococcus aureus* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa (*ATCC 25923) using the disc diffusion method. *A. marina* and *R. mucronata* harboured the most fungal endophytes that showed the highest antibacterial activity. Minimum Inhibitory Concentration (MIC) activity for the seven isolates that exhibited high antibacterial activities against the test microorganisms compared to the positive control was determined. The ethyl acetate extracts of isolates BE5, BA11, LB4 and RC6 showed significantly lower MIC activity compared to the positive control against test microorganisms ($P < 0.05$). Therefore, this study confirms that mangrove species harbour fungal isolates that have antibacterial activity and hence could serve as a novel source of antibiotics.

INTRODUCTION

Throughout thousands of years and ages, nature has been a source of medicinal agents (Petrovska, 2012). An impressive number of drugs in the modern world have been isolated from natural sources based on their use in traditional medicine (Yuan et al., 2016). Natural products remain a consistent source of drug and drug leads with several exciting molecules being reported from naturally occurring and genetically engineered microorganisms (Cragg and Newman, 2013).

A recent survey conducted by China-based research groups reported that there has been an increase in the number of scientific publications on a large proportion of discovered marine bioactive compounds of microbial origin rather than plant origin (Sun et al., 2019). The main featured microorganisms are endophytes from mangroves, marine algae, and marine invertebrates, making up more than 60% of the investigated microbial strains (Sun et al., 2019). Out of 897 publications from 2009–2018, 80% strongly focused on fungi as a potential natural source of marine bioactive compounds (Sun et al., 2019). Marine environment has been a center of focus lately since the oceans cover more than 70% of the world's surface and more than 15,000 unique natural compounds have been discovered from marine organisms (Malve, 2016). It is predicted that there are over two million marine species but only between 200,000 and 230,000 have been described (Appeltans et al., 2012). The marine invertebrates make up over 75% of the described marine species and over 95% of all marine animal species (Kemp and Baillie, 2012). This indicates that oceans offer unlimited potential for natural products and chemical diversity. Secondly, it provides quantitative habitat diversity, indicating that large populations of useful microorganisms are found in the ocean.

Further, majority of anticancer and antimicrobial drugs currently in clinical use or in development phases are natural molecules or their derivatives (Greenwell and Rahman, 2015). In comparison to other natural sources such as higher plants, microorganisms have not been greatly explored for human benefit (Jacoby et al., 2017).

Fungal endophytes are potential sources of bioactive compounds for medicinal purposes and there is a need to explore their diversity (Sarasan et al., 2017). Fungal endophytes are important to plant growth by producing enzymes and secondary metabolites, which assist in adaptation of plants to biotic stresses such as insects, herbivores, or invading pathogens and abiotic stresses such as drought and light (Isah, 2019). Plants that grow in great biodiversity also have the potential for housing endophytes with great diversity and many endophytic fungi have been found to colonize the mangrove species (Sridhar, 2012). Mangroves in special environments should be frequently studied for screening for presence of endophytes that produce antimicrobial agents (Suryanarayanan et al., 2009).

Some of the challenges to the development of therapeutics against diseases are resistance to the already existing antibiotics and hence there less toxic and more potent antibiotics are required (Davies and Davies, 2010). It is emphasized by modern researchers that there is a need to search for unexplored habitats like forests (Strobel & Daisy, 2003), marine sponges (Höller et

al., 2000) and mangroves (Lin et al., 2001) for pharmacologically active compounds. Mangrove endophytic fungi constitute the second largest group of marine endophytes (Sridhar et al., 2012) with leaves harboring a more diverse fungal endophytes community compared to other parts of the plant (Hamzah et al., 2018). Endophytic fungi have been reported to be abundant in all tissues such as flowers, fruits, stems, roots, and leaves that are potential natural sources of bioactive compounds (Rana et al., 2019). This study aimed at molecular characterization and determination of the antibacterial activity of endophytic fungi associated with selected mangrove plants of the Kenyan coast.

MATERIALS AND METHODS

Sample collection, preparation and isolation of mangrove endophytic fungi

Healthy leaves, aerial branches, and submerged roots of *Heritiera littoralis*, *Rhizophora mucronata, Bruguiera gymnorrhiza*, *Avicennia marina* and *Xylocarpus granatum* mangrove species were collected from Mida creek, Tudor creek and Gazi bay along the Kenyan coastline. Isolation of the mangrove fungal endophytic was done at the Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa.

A total of 30 fungal isolates out of 76 fungal isolates (Wacira et al., 2020)were randomly selected based on their morphological characters and identified based on the analysis of the ITS gene region. In addition, the isolates were screened for their antibacterial activity.

Molecular characterization

Genomic DNA Extraction

Approximately 100mg of fungal mycelia from 5-day old fungal pure cultures grown on Potato Dextrose Agar (PDA) at room temperature was scraped out from the culture plates using a sterile surgical blade. A Quick-DNA bacterial/ fungal kit (Zymo Research Corp. CA, United States) was used to extract the genomic DNA (gDNA) as described by the manufacturer, and the presence of the extracted gDNA was confirmed by running gel electrophoresis. Briefly, a 1% agarose gel was prepared by weighing 1-gram agarose and mixing it into 100 ml of 1x TAE buffer. The mixture was then heated to boil in a microwave and cooled down to a 50˚C. Gel electrophoresis was conducted in 1× TAE buffer at 90 units of voltage for 1 hour and visualized under ultraviolet after staining with ethidium bromide. To evaluate DNA purity, absorbance was observed from 230nm to 320nm to detect other possible contaminants (Eppendorf BioSpectrometer, Germany). The gDNA was stored at − 20°C for further use.

Polymerase Chain Reaction (PCR) amplification of the ITS gene and Sequencing

The PCR amplification of the fungal internal transcribed spacer (ITS) rDNA gene region from the gDNA was performed using ITS1 (5́TCCGTAGGTGAACCTTGCGG3´) and ITS4 (5´TCCTCCGCTTATTGATATGC 3´) primers (White et al., 1990). Amplification proceeded in a 30 cycles PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with initial heating at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 53°C for 40 seconds and extension at 72°C for 1 minute. The final elongation step was done at 72°C for 5 minutes. The presence of PCR products was confirmed using 1% agarose gel electrophoresis in 1× TAE buffer at 90 voltage for 1 hour and visualized under ultraviolet upon staining with ethidium bromide. The PCR products were purified using the QIA quick PCR purification Kit protocol (Qiagen, Germany) based on manufacturer's instructions. The purified PCR products were stored at − 20˚C before being shipped for sequencing. Sequencing of the purified PCR products was done using a commercial service provider (Inqaba Biotech,Pretoria, South Africa).

Phylogenetic analysis

The DNA sequences were trimmed and edited to obtain complete sequences using BioEdit software. A search for similar sequences using BLASTN (Altschul et al., 1990) was performed at the National Center for Biotechnology Information (NCBI) GenBank: https://www.ncbi.nlm.nih.gov/nucleotide/ (Benson et al., 2009). From the GenBank sequence database, the closest nucleotide sequences were retrieved and put onto a fasta file format that had the other newly obtained sequences from the study. Subsequently, all the sequences were aligned using the CLUSTAL Omega program (http://www.clustal.org) (Sievers and Higgins, 2017) against the nearest neighbours. A neighbor-joining tree of the aligned sequences was constructed (Saitou and Nei, 1987) using MEGA X software (Kumar et al., 2018). Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004). To obtain statistical support values for the branches, bootstrapping (Felsenstein, 1985) was conducted with 1000 replicates. All sites, including gaps in the sequence alignment, were excluded pairwise in the phylogenetic analysis. Using the resultant neighbor-joining tree, each isolate was assigned to the proper taxonomic group. The taxonomic assignment was confirmed at 90% confidence level using the naïve Bayesian rRNA classifier on the RDP website (Cole et al., 2009).

Extraction of crude extracts for antibacterial activity screening

Plugs of agar with mycelia growth covering the surface of the inoculated PDA (HiMedia, Mumbai, India) (39 g in 1 L distilled water) were transferred aseptically into 500ml Erlenmeyer flasks containing 100ml of Potato dextrose broth (TM Media, Rajasthan, India) (24 g in 1 L sterile seawater). The inoculated flasks were then incubated at 26°C both stationary and on a shaker at 200 rpm for 15 days. Cultures were harvested by filtering off the mycelium using a filter funnel and Whatman # 1 filter paper.

The broth of each flask was filtered through Whatman # 1 filter paper and the residue (mycelium) was harvested. The filtrate was extracted with 200ml ethyl acetate (EtOAc), and centrifuged at 8000 rpm for 10 minutes at room temperature and the upper layer (ethyl acetate) was collected and poured into a sterile conical flask. The extraction was repeated three times with equal volumes of EtOAc. The filtrate was also fractionated with 200ml butanol and repeated thrice. The harvested mycelia cultures were frozen at -40°C overnight, frozen-dried in a freeze dryer (ULVAC Technologies, Methuen, Japan) and the powdered material (10g) was extracted twice with ethyl acetate and butanol (Synytsya et al., 2017). The ethyl acetate and butanol extracts for both fungal broth and mycelia were then evaporated in a vacuum using a rotary evaporator (BIOBASE Company, Jiangsu, China) and dried to form an oil residue. The crude extracts were then dissolved in 1 ml Dimethyl sulphoxide (1% DMSO) for antibacterial bioassay and stored at -20°C.

Screening for antibacterial activity

The crude extracts of the broth and fungal mycelia were screened for their antibacterial activity based on Kirby–Bauer test (Hudzicki, 2012). The EtOAc and butanol extracts were tested against two gram-negative bacteria: *Escherichia coli* (ATCC 25922); *Staphylococcus aureus (*ATCC 27853) and one gram-positive bacteria *Pseudomonas aeruginosa* (ATCC 25923) using the paper disk method and each treatment comprised of three replicates. The test microorganisms were sourced from the Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. The cell suspensions of the bacteria were adjusted to approximately 10^5CFU/ml using McFarland standards.

A sterile cotton swab was dipped into the bacterial suspension. To remove excess liquid, the swab was rotated several times with firm pressure on the inside wall of the tube above the fluid level. Using the swab, the Mueller-Hinton agar plate (MHA) (HiMedia, Mumbai, India) was streaked to form a bacterial lawn. To obtain uniform growth, the plate was streaked with the swab in one direction, rotated at 90°, and streaked again in that direction. The plate was allowed to dry for approximately 5 minutes.

Each of the 6 mm sterile Whatman Antibiotic Assay Discs (Sigma-Aldrich Company, St. Louis, Germany) was impregnated with 2µL of crude extracts, negative (DMSO) or positive control (streptomycin antibiotic). In each of the test plates, 10 mg/ml in distilled water of positive control (streptomycin antibiotic), negative control (DMSO), ethyl acetate and butanol crude extracts (10 mg/ml in DMSO) were placed. The discs were placed on the surface of the medium containing 10^5 cells of bacteria test

strains and the plates were kept in the biosafety cabinet for 5 minutes to allow the agents to diffuse into the agar. After overnight incubation at 37°C, the diameter of zones of inhibition were measured in millimeters using a ruler.

Minimum Inhibitory Concentration (MIC)

The MICs of the seven most promising endophytic fungal extracts on the test isolates (*E. coli, S. aureus* and *P. aeruginosa*) were analyzed using the broth dilution method described by (Andrews, 2001). Five tubes were used; to each tube, 2 ml of sterile nutrient broth (NB) was placed in the 2nd to 5th tubes (except in tube 1). To tube 1 and 2, 2 ml of the endophytic fungal extracts (10 mg/ml) was placed and a 2-fold serial dilution was performed from the 2nd to 5th tubes, while 2 ml was discarded from the 5th tube to obtain 0.625 mg/ml, 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml concentrations. Equal volume of 0.2ml broth culture of 0.5 McFarland turbid identified bacteria was added to all the tubes dilution ranges. An overnight broth culture of 0.2 ml was placed in a positive control tube and 2 ml of NB was added to serve as control for testing the growing ability of the medium. 2ml nutrient broth was to serve as negative control for testing the sterility of medium and equipment.

The turbidity of each tube (optical density) was measured at zero hour (T_0) using a UV-VIS spectrophotometer (Konik, Barcelona, Spain) at 620nm wavelength. The tubes were incubated at 37°C for 24 hours and absorbance of each tube which is proportional to the turbidity of the bacterial growth was noted (Meletiadis et al., 2017). The MIC was defined and recorded as the lowest concentration of the test antibacterial agent that had inhibition on 50% bacterial growth. This was determined by assessing growth by calculating the difference in absorbance between the test tubes and the control tubes that had the broth and antibacterial agent alone without the test bacteria.

Data Analysis

All the experiments were carried out in triplicates. Two-way ANOVA was performed to test whether or not there were significant differences in diameter of zones of inhibition between the different extracts of fungal endophytes recovered from the mangrove species. The zones of inhibition were presented as mean ± standard deviation (SD).

Furthermore, statistical tests of means with the same letters within the column are not significantly different from one another (Fisher LSD at 95% confidence) using MINITAB. The differences were decided as significantly different with condition $p < 0.05$ ($\alpha = 0.05$).

RESULTS

Affiliation of ITS gene sequences of the isolates

A total of 30 endophytic isolates were analyzed. The isolates (with their accession numbers in parenthesis) in the inferred phylogenetic trees were affiliated with the genus *Aspergillus*, belonging to the fungal phylum Ascomycota (Fig. 1). Comparison of the newly isolated ITS gene sequences to known sequences in the GenBank database using BLASTn analysis indicated sequences similarities of > 98% with known sequences in the nucleotide sequence database (Table 1). All the isolates were affiliated with several known fungal species from the genus *Aspergillus* with > 98% sequence identity (Table 1). Ten isolates (accession numbers MW788473 to MW788478, MW788489, and MW788491 to MW788493) had between 98–100% sequences identities with known *Aspergillus* species (*Aspergillus flavus* and *Aspergillus oryzae*) and together formed a single sub-cluster supported with a bootstrap value of 98% (Fig. 1; Table 1). Out of the ten isolates, BB5 (MW788473) from Mida Creek and LB3 (MW788474) from Tudor Creek were recovered from a branch and leaf of *R. mucronata*, respectively. Isolates RA3 (MW788477) from Mida Creek and BA5 (MW788478) from Tudor Creek were recovered from a root and branch of *B. gymnorrhiza*, while isolates BD5 (MW788476) and BD2 (MW788489) were recovered from a branch of *X. granatum* from Gazi Bay. Isolates BE6 [MW788491], BC3 [MW788492] and BA7 [MW788493] were recovered from branches of *H. littoralis, A. marina* and *B. gymnorrhiza*, respectively (Table 1).

Nine isolates (BE5 (MW788480), BB8 (MW788482), BB2 (MW788483), BD4 (MW788484), RA4 (MW788485), RC7 (MW788486), BE3 (MW788487), BB10 (MW788488) and BC10 (MW788490)) had > 98% sequence identities with *Aspergillus niger* (Table 1)

and together formed a minor sub-cluster supported with a bootstrap value of 92% (Fig. 1). Out of the nine, three isolates (BB8 (MW788482), BB2 (MW788483) and BB10 (MW788488) were obtained from branches of *R. mucronata.* Two isolates (RC7 (MW788486) and BC10 (MW788490)) were recovered from a root and branch of *A. marina*, while isolates BE5 (MW788480) and BE3 (MW788487) were recovered from branches of *H. littoralis.* Isolates BD4 (MW788484) and RA4 (MW788485) were recovered from *X. granatum* and *B. gymnorrhiza*, respectively (Table 1). Within the same sub-cluster, isolate BA1 (MW788481), which was recovered from a branch of *B. gymnorrhiza*, formed a minor sub-cluster with *Aspergillus tubingensis* (KX664401) (Fig. 1).

Two isolates namely (BC9 (MW788479) and BC5 (MW788479)) were closely related to *Aspergillus assiutensis* with 99% sequence identity and together formed a separate sub-cluster supported with a bootstrap value of 100% (Fig. 1). These isolates (BC9 and BC5) were both obtained from the branches of *A. marina* from Mida creek (Table 1).

Table 1
Affiliation of endophytic fungi with their closest taxonomic relatives and their associated host mangrove species.

Sample ID	Accession No.	Host mangrove species	Location	Closest taxonomic affiliation	Isolation Source	Country	% ID
BB5	MW788473	*R. mucronata*	Mida creek	*Aspergillus* sp. [MN844036]	Rubia plant endophyte (Meletiadis et al., 2017)	China	99
LB3	MW788474	*R. mucronata*	Tudor creek	*Aspergillus flavus* [MT558941]	Rosy rice vinegar solid mash (Meletiadis et al., 2017)	India	100
BC8	MW788475	A. *marina*	Tudor creek	*Aspergillus flavus* [MT645322]	Insect larva (Meletiadis et al., 2017)	China	100
BD5	MW788476	*X. granatum*	Gazi Bay	*Aspergillus oryzae* [MG437005]	Coastal marine sediment (Meletiadis et al., 2017)	India	100
RA3	MW788477	*B. gymnorrhiza*	Mida creek	*Aspergillus flavus* [MT558941]	Rosy rice vinegar solid mash (Meletiadis et al., 2017)	India	100
BA5	MW788478	*B. gymnorrhiza*	Tudor creek	*Aspergillus* sp. [MH341160]	Animal Leather (Meletiadis et al., 2017)	Pakistan	99
BC9	MW788479	*A. marina*	Mida creek	*Aspergillus aculeatus* [MN187974]	Marine Estuary Tamil Nadu (Meletiadis et al., 2017)	India	99
BE5	MW788480	*H. littoralis*	Gazi Bay	*Aspergillus niger* [MK534501]	Leachate contaminated soil (Meletiadis et al., 2017)	Malaysia	100
BA1	MW788481	*B. gymnorrhiza*	Mida creek	*Aspergillus tubingensis* [KX664401]	Floor surface (Meletiadis et al., 2017)	USA	99
BB8	*MW788482*	*R. mucronata*	Gazi Bay	*Aspergillus niger* [KJ410675]	Soil from Stevia plants (Meletiadis et al., 2017)	India	100
BB2	*MW788483*	*R. mucronata*	Mida creek	*Aspergillus niger* [MT620753]	Rice Wine (Meletiadis et al., 2017)	China	100
BD4	*MW788484*	*X. granatum*	Gazi Bay	*Aspergillus niger* [MT588793]	Plant (Meletiadis et al., 2017)	India	100
RA4	*MW788485*	*B. gymnorrhiza*	Mida creek	*Aspergillus niger* [MT620753]	Rice Wine (Meletiadis et al., 2017)	China	100
RC7	*MW788486*	*A. marina*	Mida creek	*Aspergillus niger* [MT588793]	Plant (Meletiadis et al., 2017)	India	100
BE3	*MW788487*	*H. littoralis*	Gazi Bay	*Aspergillus niger* [MK534501]	Leachate contaminated soil (Meletiadis et al., 2017)	Malaysia	100
BB10	*MW788488*	*R. mucronata*	Tudor creek	*Aspergillus niger* [MT550026]	N/A	Thailand	100
BD2	*MW788489*	*X. granatum*	Gazi Bay	*Aspergillus oryzae* [MT558944]	Zhejiang rosy rice vinegar (Meletiadis et al., 2017)	China	100
BC10	MW788490	*R. mucronata*	Gazi Bay	*Aspergillus niger* [MT588793]	Soil from Stevia plants (Meletiadis et al., 2017)	India	99
BE6	*MW788491*	*H. littoralis*	Gazi Bay	*Aspergillus oryzae* [MT558944]	Zhejiang rosy rice vinegar (Meletiadis et al., 2017)	China	100

Sample ID	Accession No.	Host mangrove species	Location	Closest taxonomic affiliation	Isolation Source	Country	% ID
BC3	*MW788492*	*A. marina*	Tudor creek	*Aspergillus oryzae* [MT558944]	Zhejiang rosy rice vinegar (Meletiadis et al., 2017)	China	100
BA7	*MW788493*	*B. gymnorrhiza*	Tudor creek	*Aspergillus flavus* [MT584825]	N/A	China	100
BC5	MW788494	*A. marina*	Mida creek	*Aspergillus aculeatus* [MN187297]	Marine Estuary (Meletiadis et al., 2017)	India	99

Screening for antibacterial activity

The fungal broth and mycelia were tested against three human pathogenic strains of Gram-positive bacteria (*S. aureus* (ATCC 27853) and Gram-negative bacteria (*Escherichia coli* (ATCC 25922) and *P. aeruginosa (*ATCC 25923) (Tables 2, 3 and 4).

Seven fungal broth crude extracts (LB4, BD4, BE5, BA11, RC6, RC3 and LB1) out of the twenty-nine exhibited high antibacterial activities against the three tested microorganisms and were more effective than the positive control (Tables 2 and 3).

The ethyl acetate extracts of the endophytic fungal isolates LB4, BD4, BE5 and BA11 exhibited high antibacterial activities against all the test microorganisms compared to the positive control (Table 2). The extracts of RC6 (29.17 ± 0.1 mm 0.1 mm) and RC3 (29.17 ± 0.1 mm) showed a wide variety of antibacterial activities against *P. aeruginosa* (ATCC 25923) compared to the positive control.

The ethyl acetate extracts of LB4 (41.03 ± 0.1), BD4 (39.07 ± 0.1), BE5 (34.03 ± 0.1) and BA11 (36.03 ± 0.1) showed a comparable higher antibacterial activity against *E. coli* (ATCC 25922) than the positive control (Table 2). The inhibitory activity of the ethyl acetate extracts against *P. aeruginosa* (ATCC 25923) was observed as LB4 (35.07 ± 0.1 mm), BD4 (35.07 ± 0.1 mm), BE5 (32.13 ± 0.1 mm) and BA11 (39.03 ± 0.1 mm) compared to the positive control.

A wide range of antibacterial activity was exhibited by ethyl acetate extracts of LB4 (44.07 ± 0.1 mm), BD4 (31.07 ± 0.1 mm), BE5 (35.07 ± 0.1 mm) and BA11 (39.03 ± 0.1 mm) against *S. aureus* (ATCC 27853) compared to the positive control.

Butanol extracts of isolate LB1 (29.07 ± 0.1 mm) showed a wide range of antibacterial activities against *P. aeruginosa* (ATCC 25923) compared to the positive control (28.30 ± 0.2 mm) (Table 3).

In the fungal mycelia, ethyl acetate extract of sample M2 was more effective in *S. aureus* (ATCC 27853) compared to other extracts (Table 4). Butanol extract of sample M8 was effective in *S. aureus* (ATCC 27853) and *E. coli* (ATCC 25922) but not in *P. aeruginosa* (ATCC 25923).

Note that ethyl acetate extracts of isolate BB6 recovered from *R. mucronata* (Table 2) and butanol extracts of isolates RA3 and BA6 from *B. gymnorrhiza* of Mida Creek (Table 3) showed no antibacterial activities against all test microorganisms. From this study work, ethyl acetate was the most effective solvent compared with butanol ($p < 0.05$).

Table 2
Antibacterial activities of ethyl acetate extracts from mangrove endophytic fungi.

Sample ID	Endophytic fungal isolate	Site sampled	Host plant	Tissue type	Ethyl acetate extracts inhibition zone diameters (mm)		
					Gram-negative bacteria		Gram-positive bacteria
					Escherichia coli (ATCC 25922)	*Pseudomonas aeruginosa* (ATCC 27853)	*Staphylococcus aureus* (ATCC 25923)
LA1	*Alternaria*	Mida creek	*B. gymnorrhiza*	L	0.00 ± 0.0^{f}	7.03 ± 0.1^{d}	14.07 ± 0.1^{abcd}
LB1	*Alternaria*	Mida creek	*R. mucronata*	L	7.07 ± 0.1^{ef}	8.17 ± 0.2^{cd}	10.30 ± 0.1^{bcd}
BB3	*Alternaria*	Tudor creek	*R. mucronata*	B	19.03 ± 0.1^{bcde}	19.17 ± 0.2^{bcd}	11.03 ± 0.1^{bcd}
BB6	*Alternaria*	Mida creek	*R. mucronata*	B	0.00 ± 0.0^{f}	0.00 ± 0.0^{d}	0.00 ± 0.0^{d}
	Negative control				0.00 ± 0.0^{f}	0.00 ± 0.0^{d}	0.00 ± 0.0^{d}
	Positive control				28.10 ± 0.1^{bcde}	28.30 ± 0.2^{abc}	31.70 ± 0.1^{abc}
LC3	*Penicillium*	Mida creek	*A. marina*	L	0.00 ± 0.0^{f}	8.03 ± 0.1^{cd}	9.07 ± 0.1^{bcd}
LC5	*Penicillium*	Tudor creek	*A. marina*	L	10.13 ± 0.1^{def}	0.00 ± 0.0^{d}	14.07 ± 0.1^{abcd}
RC6	*Penicillium*	Mida creek	*A. marina*	R	14.10 ± 0.1^{def}	29.17 ± 0.1^{abc}	26.07 ± 0.1^{abcd}
	Negative control				0.00 ± 0.0^{f}	0.00 ± 0.0^{d}	0.00 ± 0.0^{d}
	Positive control				32.20 ± 0.1^{abcd}	27.09 ± 0.1^{abc}	37.10 ± 0.1^{ab}
LB2	*Cladosporium*	Mida creek	*R. mucronata*	L	20.03 ± 0.1^{bcde}	7.03 ± 0.1^{d}	0.00 ± 0.0^{d}
LB4	*Cladosporium*	Tudor creek	*R. mucronata*	L	41.03 ± 0.1^{a}	35.07 ± 0.1^{ab}	44.07 ± 0.1^{a}
RA2	*Cladosporium*	Mida creek	*B. gymnorrhiza*	R	0.00 ± 0.0^{f}	10.03 ± 0.1^{cd}	0.00 ± 0.0^{d}
	Negative control				0.00 ± 0.0^{f}	0.00 ± 0.0^{d}	0.00 ± 0.0^{d}
	Positive control				35.40 ± 0.1^{abc}	28.07 ± 0.1^{abc}	37.40 ± 0.1^{ab}
RA3	*Aspergillus*	Mida creek	*B. gymnorrhiza*	R	26.07 ± 0.1^{bcde}	29.07 ± 0.1^{abc}	22.10 ± 0.1^{abcd}

Sample ID	Endophytic fungal isolate	Site sampled	Host plant	Tissue type	Ethyl acetate extracts inhibition zone diameters (mm)		
					Gram-negative bacteria		Gram-positive bacteria
					Escherichia coli (ATCC 25922)	*Pseudomonas aeruginosa* (ATCC 27853)	*Staphylococcus aureus* (ATCC 25923)
BA5	*Aspergillus*	Tudor creek	*B. gymnorrhiza*	B	9.03 ± 0.1^{ef}	10.17 ± 0.1^{cd}	14.07 ± 0.1^{abcd}
BA6	*Aspergillus*	Mida creek	*B. gymnorrhiza*	B	6.03 ± 0.1^{ef}	10.67 ± 0.1^{cd}	0.00 ± 0.0^{d}
BB8	*Aspergillus*	Gazi bay	*R. mucronata*	B	0.00 ± 0.0^{f}	19.03 ± 0.1^{bcd}	15.03 ± 0.1^{abcd}
BC3	*Aspergillus*	Gazi bay	*A. marina*	B	10.67 ± 0.1^{def}	11.03 ± 0.1^{cd}	8.07 ± 0.1^{bcd}
BC8	*Aspergillus*	Tudor creek	*A. marina*	B	14.10 ± 0.1^{def}	8.07 ± 0.1^{cd}	9.03 ± 0.1^{bcd}
BC9	*Aspergillus*	Mida creek	*A. marina*	B	10.03 ± 0.1^{def}	10.03 ± 0.1^{cd}	13.07 ± 0.1^{bcd}
BD4	*Aspergillus*	Gazi bay	*X. granatum*	B	39.07 ± 0.1^{ab}	35.07 ± 0.1^{ab}	31.07 ± 0.1^{abc}
BE5	*Aspergillus*	Gazi bay	*H. littoralis*	B	34.03 ± 0.1^{abc}	32.13 ± 0.1^{ab}	35.07 ± 0.1^{abc}
	Negative control				0.00 ± 0.0^{f}	0.00 ± 0.0^{d}	0.00 ± 0.0^{d}
	Positive control				28.30 ± 0.1^{bcde}	29.30 ± 0.4^{abc}	29.20 ± 0.3
BA10	*Fusarium*	Gazi bay	*B. gymnorrhiza*	B	0.00 ± 0.0^{f}	12.07 ± 0.1^{cd}	10.07 ± 0.1^{bcd}
BC6	*Fusarium*	Mida creek	*A. marina*	B	0.00 ± 0.0^{f}	0.00 ± 0.0^{d}	11.03 ± 0.1^{bcd}
	Negative control				0.00 ± 0.0^{f}	0.00 ± 0.0^{d}	0.00 ± 0.0^{d}
	Positive control				21.03 ± 0.1^{bcde}	24.10 ± 0.1^{abc}	28.60 ± 0.1^{abcd}
RB4	*Lasiodiplodia*	Mida creek	*R. mucronata*	R	0.00 ± 0.0^{f}	10.07 ± 0.1^{cd}	15.07 ± 0.1^{abcd}
BB4	*Lasiodiplodia*	Tudor creek	*R. mucronata*	B	9.03 ± 0.1^{ef}	8.03 ± 0.1^{cd}	18.07 ± 0.1^{abcd}
BB7	*Lasiodiplodia*	Mida creek	*R. mucronata*	B	16.03 ± 0.1^{def}	25.10 ± 0.1^{abc}	21.13 ± 0.1^{abcd}
	Negative control				0.00 ± 0.0^{f}	0.00 ± 0.0^{d}	0.00 ± 0.0^{d}

Sample ID	Endophytic fungal isolate	Site sampled	Host plant	Tissue type	Ethyl acetate extracts inhibition zone diameters (mm)		
					Gram-negative bacteria		Gram-positive bacteria
					Escherichia coli (ATCC 25922)	*Pseudomonas aeruginosa* (ATCC 27853)	*Staphylococcus aureus* (ATCC 25923)
	Positive control				25.70 ± 0.1^{bcde}	30.10 ± 0.1^{abc}	39.70 ± 0.1^{a}
BA8	*Nigrospora*	Mida creek	*B. gymnorrhiza*	B	0.00 ± 0.0^{f}	9.03 ± 0.1^{cd}	9.03 ± 0.1^{bcd}
BA11	*Nigrospora*	Tudor creek	*B. gymnorrhiza*	B	36.03 ± 0.1^{abc}	39.03 ± 0.1^{a}	39.03 ± 0.1^{a}
BC7	*Nigrospora*	Mida creek	*A. marina*	B	11.03 ± 0.1^{def}	0.00 ± 0.0 ^{d}	0.00 ± 0.0^{d}
RC3	*Nigrospora*	Mida creek	*A. marina*	R	26.07 ± 0.1^{bcde}	29.17 ± 0.1^{abc}	22.03 ± 0.1^{abcd}
	Negative control				0.00 ± 0.0^{f}	0.00 ± 0.0^{d}	0.00 ± 0.0^{d}
	Positive control				34.60 ± 0.1^{abc}	27.00 ± 0.1^{abc}	30.10 ± 0.1^{abc}
BC2	*Chaetomium*	Gazi bay	*A. marina*	B	10.07 ± 0.1^{def}	19.03 ± 0.1^{cd}	17.03 ± 0.1^{abcd}
	Negative control				0.00 ± 0.0^{f}	0.00 ± 0.0^{d}	0.00 ± 0.0^{d}
	Positive control				25.03 ± 0.1^{bcde}	25.03 ± 0.1^{abc}	28.07 ± 0.1^{abcd}

The isolates are coded depending on the part of the tree sampled; the prefix letters L, B, and R representing leaf, branch, and root tissue of the mangrove species respectively. Then followed by the letters (A, B, C, D, and E) representing *B. gymnorrhiza, R. mucronata, A. marina, X. granatum* and *H. littoralis* mangrove species, respectively, and the isolate number of the sample collected from the mangrove species. There was significant difference between gram (-ve) and, gram (+ ve) inhibition ($p < 0.05$ ($\alpha = 0.05$)).

Table 3
Antibacterial activities of butanol extracts from mangrove endophytic fungi.

Sample ID	Endophytic fungal isolate	Site sampled	Host plant	Tissue type	Butanol extracts inhibition zone diameters (mm)		
					Gram-negative bacteria		Gram-positive bacteria
					Escherichia coli (ATCC 25922)	*Pseudomonas aeruginosa* (ATCC 27853)	*Staphylococcus aureus* (ATCC 25923)
LA1	*Alternaria*	Mida creek	*B. gymnorrhiza*	L	9.03 ± 0.1^{ab}	10.13 ± 0.2^{ab}	9.03 ± 0.1^{bcd}
LB1	*Alternaria*	Mida creek	*R. mucronata*	L	10.13 ± 0.1^{ab}	29.07 ± 0.1^{a}	10.07 ± 0.1^{abcd}
BB3	*Alternaria*	Tudor creek	*R. mucronata*	B	7.07 ± 0.1^{ab}	19.17 ± 0.1^{a}	6.07 ± 0.1^{cd}
BB6	*Alternaria*	Mida creek	*R. mucronata*	B	10.03 ± 0.1^{ab}	12.07 ± 0.1^{ab}	10.13 ± 0.1^{abcd}
	Negative control				0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	0.00 ± 0.0^{e}
	Positive control				28.10 ± 0.1	28.30 ± 0.2	31.70 ± 0.1
LC3	*Penicillium*	Mida creek	*A. marina*	L	0.00 ± 0.0^{b}	10.13 ± 0.1^{ab}	10.03 ± 0.1^{abcd}
LC5	*Penicillium*	Tudor creek	*A. marina*	L	12.07 ± 0.1^{ab}	8.07 ± 0.1^{ab}	10.10 ± 0.1^{abcd}
RC6	*Penicillium*	Mida creek	*A. marina*	R	0.00 ± 0.0^{b}	8.03 ± 0.1^{ab}	0.00 ± 0.0^{e}
	Negative control				0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	0.00 ± 0.0^{e}
	Positive control				32.20 ± 0.1	27.09 ± 0.1	37.10 ± 0.1
LB2	*Cladosporium*	Mida creek	*R. mucronata*	L	10.23 ± 0.1^{ab}	8.03 ± 0.1^{ab}	12.03 ± 0.1^{abc}
LB4	*Cladosporium*	Tudor creek	*R. mucronata*	L	15.07 ± 0.1^{ab}	10.07 ± 0.1^{ab}	8.07 ± 0.1^{abcde}
RA2	*Cladosporium*	Mida creek	*B. gymnorrhiza*	R	7.17 ± 0.1^{ab}	7.07 ± 0.1^{ab}	8.07 ± 0.1^{abcde}
	Negative control				0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	0.00 ± 0.0^{e}
	Positive control				35.40 ± 0.1	28.07 ± 0.1	37.40 ± 0.1
RA3	*Aspergillus*	Mida creek	*B. gymnorrhiza*	R	0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	0.00 ± 0.0^{e}

Sample ID	Endophytic fungal isolate	Site sampled	Host plant	Tissue type	Butanol extracts inhibition zone diameters (mm)		
					Gram-negative bacteria		Gram-positive bacteria
					Escherichia coli (ATCC 25922)	*Pseudomonas aeruginosa* (ATCC 27853)	*Staphylococcus aureus* (ATCC 25923)
BA5	*Aspergillus*	Tudor creek	*B. gymnorrhiza*	B	10.03 ± 0.1^{ab}	10.03 ± 0.1^{ab}	10.03 ± 0.1^{abcd}
BA6	*Aspergillus*	Mida creek	*B. gymnorrhiza*	B	0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	0.00 ± 0.0^{e}
BB8	*Aspergillus*	Gazi bay	*R. mucronata*	B	0.00 ± 0.0^{b}	10.07 ± 0.1^{ab}	19.07 ± 0.1^{a}
BC3	*Aspergillus*	Gazi bay	*A. marina*	B	8.03 ± 0.1^{ab}	9.03 ± 0.1^{ab}	9.03 ± 0.1^{bcd}
BC8	*Aspergillus*	Tudor creek	*A. marina*	B	9.03 ± 0.1^{ab}	8.07 ± 0.1^{ab}	9.03 ± 0.1^{bcd}
BC9	*Aspergillus*	Mida creek	*A. marina*	B	0.00 ± 0.0^{b}	9.03 ± 0.1^{ab}	0.00 ± 0.0^{e}
BD4	*Aspergillus*	Gazi bay	*X. granatum*	B	11.03 ± 0.1^{ab}	8.03 ± 0.1^{ab}	9.03 ± 0.1^{bcd}
BE5	*Aspergillus*	Gazi bay	*H. littoralis*	B	17.03 ± 0.1^{a}	16.03 ± 0.1^{a}	21.23 ± 0.3^{a}
	Negative control				0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	0.00 ± 0.0^{e}
	Positive control				28.30 ± 0.1	29.30 ± 0.4	29.20 ± 0.3
BA10	*Fusarium*	Gazi bay	*B. gymnorrhiza*	B	8.03 ± 0.1^{ab}	8.03 ± 0.1^{ab}	9.03 ± 0.1^{bcd}
BC6	*Fusarium*	Mida creek	*A. marina*	B	7.17 ± 0.1^{ab}	10.07 ± 0.1^{ab}	12.07 ± 0.1^{abc}
	Negative control				0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	0.00 ± 0.0^{e}
	Positive control				21.03 ± 0.1	24.10 ± 0.1	28.60 ± 0.1
RB4	*Lasiodiplodia*	Mida creek	*R. mucronata*	R	8.03 ± 0.1^{ab}	10.07 ± 0.1^{ab}	11.03 ± 0.1^{abc}
BB4	*Lasiodiplodia*	Tudor creek	*R. mucronata*	B	19.03 ± 0.1^{ab}	7.07 ± 0.1^{ab}	19.03 ± 0.1^{a}
BB7	*Lasiodiplodia*	Mida creek	*R. mucronata*	B	0.00 ± 0.0^{b}	9.03 ± 0.1^{ab}	18.07 ± 0.1^{ab}
	Negative control				0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	0.00 ± 0.0^{e}

Sample ID	Endophytic fungal isolate	Site sampled	Host plant	Tissue type	Butanol extracts inhibition zone diameters (mm)		
					Gram-negative bacteria		Gram-positive bacteria
					Escherichia coli (ATCC 25922)	*Pseudomonas aeruginosa* (ATCC 27853)	*Staphylococcus aureus* (ATCC 25923)
	Positive control				25.70 ± 0.1	30.10 ± 0.1	39.70 ± 0.1
BA8	*Nigrospora*	Mida creek	*B. gymnorrhiza*	B	10.07 ± 0.1^{ab}	12.07 ± 0.1^{ab}	7.07 ± 0.1^{bcde}
BA11	*Nigrospora*	Tudor creek	*B. gymnorrhiza*	B	15.07 ± 0.1^{ab}	26.07 ± 0.1^{a}	20.03 ± 0.1^{a}
BC7	*Nigrospora*	Mida creek	*A. marina*	B	11.03 ± 0.1^{ab}	10.07 ± 0.1^{ab}	10.07 ± 0.1^{abcd}
RC3	*Nigrospora*	Mida creek	*A. marina*	R	0.00 ± 0.0^{b}	16.03 ± 0.1^{a}	10.07 ± 0.1^{abcd}
	Negative control				0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	0.00 ± 0.0^{e}
	Positive control				34.60 ± 0.1	27.00 ± 0.1	30.10 ± 0.1
BC2	*Chaetomium*	Gazi bay	*A. marina*	B	9.03 ± 0.1^{ab}	11.03 ± 0.1^{ab}	10.03 ± 0.1^{abcd}
	Negative control				0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	0.00 ± 0.0^{e}
	Positive control				25.03 ± 0.1	25.03 ± 0.1	28.07 ± 0.1

The isolates are coded depending on the part of the tree sampled; the prefix letters L, B, and R representing leaf, branch, and root tissue of the mangrove species respectively. Then followed by the letters (A, B, C, D, and E) representing *B. gymnorrhiza, R. mucronata, A. marina, X. granatum* and *H. littoralis* mangrove species, respectively, and the isolate number of the sample collected from the mangrove species. There was significant difference between gram (-ve) and, gram (+ ve) inhibition ($p < 0.05$ ($\alpha = 0.05$)).

Table 4
Antibacterial activities of Ethyl acetate and Butanol extracts of fungal mycelia.

ENDOPHYTIC FUNGI	Ethyl acetate and Butanol extracts inhibition zone diameters (mm)					
	Gram-negative bacteria				Gram-positive bacteria	
	Escherichia coli (ATCC 25922)		*Pseudomonas aeruginosa (ATCC 27853)*		Staphylococcus aureus (ATCC 25923)	
	EXTRACTS					
	Ethyl acetate	Butanol	Ethyl acetate	Butanol	Ethyl acetate	Butanol
Negative control	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
M1	10.07 ± 0.1^{a}	10.07 ± 0.1^{b}	11.03 ± 0.1^{a}	10.07 ± 0.1^{a}	15.07 ± 0.1^{a}	14.07 ± 0.1^{a}
M2	12.07 ± 0.1^{a}	10.07 ± 0.1^{b}	15.07 ± 0.1^{a}	15.07 ± 0.1^{a}	18.07 ± 0.1^{a}	16.03 ± 0.1^{a}
M3	12.07 ± 0.1^{a}	10.07 ± 0.1^{b}	9.03 ± 0.1^{a}	11.03 ± 0.1^{a}	15.07 ± 0.1^{a}	15.07 ± 0.1^{a}
M4	9.03 ± 0.1^{a}	10.07 ± 0.1^{b}	12.07 ± 0.1^{a}	12.07 ± 0.1^{a}	15.07 ± 0.1^{a}	12.07 ± 0.1^{a}
M5	16.03 ± 0.1^{a}	11.03 ± 0.1^{b}	11.03 ± 0.1^{a}	8.03 ± 0.1^{a}	11.03 ± 0.1^{a}	9.03 ± 0.1^{a}
M6	10.07 ± 0.1^{a}	14.07 ± 0.1^{a}	9.03 ± 0.1^{a}	10.07 ± 0.1^{a}	10.07 ± 0.1^{a}	13.07 ± 0.1^{a}
M7	0.00 ± 0.0^{a}	9.03 ± 0.1^{b}	9.03 ± 0.1^{a}	10.07 ± 0.1^{a}	10.07 ± 0.1^{a}	9.03 ± 0.1^{a}
M8	12.07 ± 0.1^{a}	16.03 ± 0.1^{a}	16.03 ± 0.1^{a}	10.07 ± 0.1^{a}	15.07 ± 0.1^{a}	20.03 ± 0.1^{a}
Positive control	28.06 ± 3.4	28.06 ± 3.4	29.15 ± 3.9	29.15 ± 3.9	33.40 ± 2.3	33.40 ± 2.3

Note that ethyl acetate extracts of isolate BB6 recovered from *R. mucronata* and butanol extracts of isolates RA3 and BA6 from *B. gymnorrhiza* of Mida Creek showed no antibacterial activities against all test microorganisms (Tables 2 and 3). From this study work, ethyl acetate was the most effective solvent compared with butanol ($p < 0.05$).

Minimum Inhibitory Concentration (MIC)

The varying concentrations between 0.625 mg/ml and 10 mg/ml of the endophytic fungal extracts were tested to determine their MICs (Table 5). The lowest MIC (2.86 ± 0.01), ethyl acetate extract of isolate BE5 was observed against *E. coli*. The butanol extract of isolate LB1 showed the least MIC activity (4.57 ± 0.01) against *P. aeruginosa.* The Ethyl acetate extracts of isolates BE5, BA11, LB4 and RC6 showed significantly lower MIC activity (Table 5) compared to the standard drug/control (streptomycin) against test microorganisms ($P < 0.05$). The ethyl acetate extracts of isolate BE5 had a MIC of 2.86 ± 0.01 compared to the control (3.77 ± 0.00) against *E. coli.* The MIC (3.05 ± 0.01) for the ethyl acetate extracts of isolate BA11 against *S. aureus* was lower than the positive control (4.5 ± 0.00). For the isolate LB4, the minimum inhibitory concentrations of 3.23 ± 0.01 and 3.69 ± 0.01 were observed against *P. aeruginosa* and *S. aureus* respectively compared to the positive controls that had minimum inhibitory concentrations of 3.84 ± 0.00 and 4.5 ± 0.00, respectively. The MIC (3.31 ± 0.01) for isolate RC6 was observed compared to the positive control (3.84 ± 0.00) against *P. aeruginosa.*

Table 5
Minimum inhibitory concentrations (MIC) of ethyl acetate and butanol extracts of the fungal isolates against the tested human pathogenic bacteria.

			MIC (mg/ml)		
No.	SAMPLE ID	EXTRACT	Escherichia coli	Pseudomonas aeruginosa	Staphylococcus aureus
1	BD4	Ethyl acetate	3.88 ± 0.01^{b}	4.96 ± 0.01^{b}	4.90 ± 0.00^{b}
2	BA11		5.32 ± 0.01^{a}	4.62 ± 0.01^{b}	3.05 ± 0.01^{c}
		Butanol	-	4.96 ± 0.01^{b}	-
3	BE5	Ethyl acetate	2.86 ± 0.01^{d}	5.17 ± 0.01^{a}	2.96 ± 0.01^{d}
4	LB4		4.94 ± 0.01^{a}	3.23 ± 0.01^{c}	3.69 ± 0.01^{c}
5	RC6	Ethyl acetate	-	3.31 ± 0.01^{c}	-
6	RC3		-	4.58 ± 0.01^{b}	-
7	LB1	Butanol	-	4.57 ± 0.01^{b}	-
CONTROL (Streptomycin)			3.77 ± 0.00^{b}	3.84 ± 0.00^{c}	4.5 ± 0.00^{b}

The isolates are denoted as LB4 and LB1 (leaf isolates of *R. mucronata*); RC6 and RC3 (root isolates of *A. marina*); and BD4, BA11, and BE5 (branch isolates of *X. granatum, B. gymnorrhiza and H. littoralis* species); '– 'denotes those values not determined. Data are presented as means ± SD of three replicates. Values with the different superscript in the same column are significantly different (P < 0.05). There was significant difference between gram *(-*ve) and gram (+ ve) inhibition *(p* < 0.05 (α = 0.05)). *Concentration of positive control (Streptomycin) is 20 mg/ml in distilled water.

DISCUSSION

A total of 30 fungal isolates were randomly selected as morphotypes of genus *Aspergillus* according to (Arnold et al., 2000) since they were the most dominant isolates in all the five-mangrove species.

This study revealed that *Aspergillus niger*, is the predominant fungal species associated with the *B. gymnorrhiza, H. littoralis, A. marina, R. mucronata* and X. granatum mangrove species. A similar study along the Coast of South Andaman Sea, Andaman and Nicobar Islands, India reported that *A. niger* was found to be prominent with a trend of rapid growth (Thorati et al., 2016). Other *Aspergillus* species reported in this study were *Aspergillus flavus, Aspergillus oryzae, Aspergillus aculeatus, Aspergillus tubingensis, Aspergillus assiutensis* and several unidentified *Aspergillus* sp.

The phylogenetic analysis revealed three main clusters that were represented by the following sections of the aspergilli; *A. niger, A. flavus, A. oryzae A. aculeatus, A. tubingensis* and *A. assiutensis*.

One cluster comprised of *A. oryzae* and *A. flavus* that confirmed the evolutionary closeness between the two species. The close genetic relatedness between *A. oryzae* and *A. flavus* was also recorded by (Arnold et al., 2000). A study on the diversity of fungi from mangroves of Mahanadi delta, Orissa, India found *A. oryzae, A. flavus* and *A. niger* were common in the area (Arnold et al., 2000).

Another main cluster consisted of the filamentous black aspergilli in the Nigri section. Within this cluster was a minor sub-cluster, which comprised of *A. niger*, *A. tubingensis* and *A.* sp. BA1 [MW788481]. *A. niger* and *A. tubingensis* are both classified as biseriate species (Arnold et al., 2000).

A. niger was found in abundance compared to the other species recorded in this study.

The last main cluster consisted of *A. assiutensis* and *A. aculeatus*. *A. aculeatus* and *A. niger* were previously found in the soil of grapevine plantations (Abdel-Sater et al., 2016). *A. assiutensis* was reported in ElKhawaled village, Sahel-Saleem city from air of grapevine plantation by (Ah et al., 2022).

The results on antibacterial activity showed that ethyl acetate was the most effective solvent compared to butanol. The endophytic fungal extracts of isolates LB4, BD4, BE5, BA11, RC6, RC3 and LB1 exhibited high antibacterial activities against the test microorganisms compared to the positive control. Among the five-mangrove species sampled, root isolates of *A. marina* (RC6 and RC3) and leaf isolates of *R. mucronata* (LB4 and LB1) had the most effective extracts against the test microorganisms compared to the positive control. Antibacterial activity of leaf extract of mangroves including *R. mucronata* from Chorao Island, Goa was investigated against human bacterial pathogens and it was reported to be a potential source for the development of novel antibiotics (Sahoo et al., 2012). Ethyl acetate root extracts of *A. marina* have also been found in the Kingdom of Saudi Arabia and observed to have antibacterial activities against *E. coli, S. aureus* and *P. aeruginosa* (Okla et al., 2021).

From this study, ethyl acetate and butanol broth extracts isolated from leaves found in Mida creek were the most effective against S. *aureus* and *P. aeruginosa* respectively compared to all the other isolates. Fungal extracts isolated from the mangrove leaves in Luzon Island, Philippines, showed antibacterial activity against the gram-positive bacteria *S. aureus.* (Ramirez et al., 2020). Both ethyl acetate and butanol mycelial extracts were most effective against *S. aureus.* Synytsya et al., 2017,observed that extracts from the fungal mycelia had antibacterial property.

The Ethyl acetate extracts of isolates BE5, BA11, LB4 and RC6 that showed a significantly lower MIC activity compared to the standard drug/control (streptomycin) against test microorganisms. Therefore, branch isolates of *X. granatum* and *B. gymnorrhiza* species; leaf isolate of *R. mucronata* species, and root isolate of *A. marina* species had more inhibitory effects on the tested microorganisms.

CONCLUSION

From this study, it is concluded that *Aspergillus niger* were the predominant fungal endophytes associated with the *R. mucronata, B. gymnorrhiza, A. marina, X. granatum and H. littoralis mangrove species.* It is also evident that genus *Aspergillus* has a high adaptability to various ecological environments and cytotoxic studies to determine if the extracts are safe would be useful. Some fungal isolates have antibacterial activity and hence results generated form the basis of future efforts on drug discovery.

Declarations

DATA AVAILABILITY STATEMENT

The data presented in this study are available upon request from the corresponding authors.

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Competing Interests

The authors have not declared any conflict of interests.

Author Contributions

Conceptualization, Teresia Nyambura Wacira, Huxley Mae Makonde, Carren Moraa Bosire and Cromwell Mwiti Kibiti; writing—original draft preparation, Teresia Nyambura Wacira; writing—review and editing, Teresia Nyambura Wacira, Huxley Mae Makonde, Carren Moraa Bosire and Cromwell Mwiti Kibiti. All authors have read and agreed to publish the manuscript.

Ethics approval

The National Commission for Science, Technology, and Innovation (NACOSTI) granted its approval for this study (approval number: TUM ERC PHD/002/2020).

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Figures

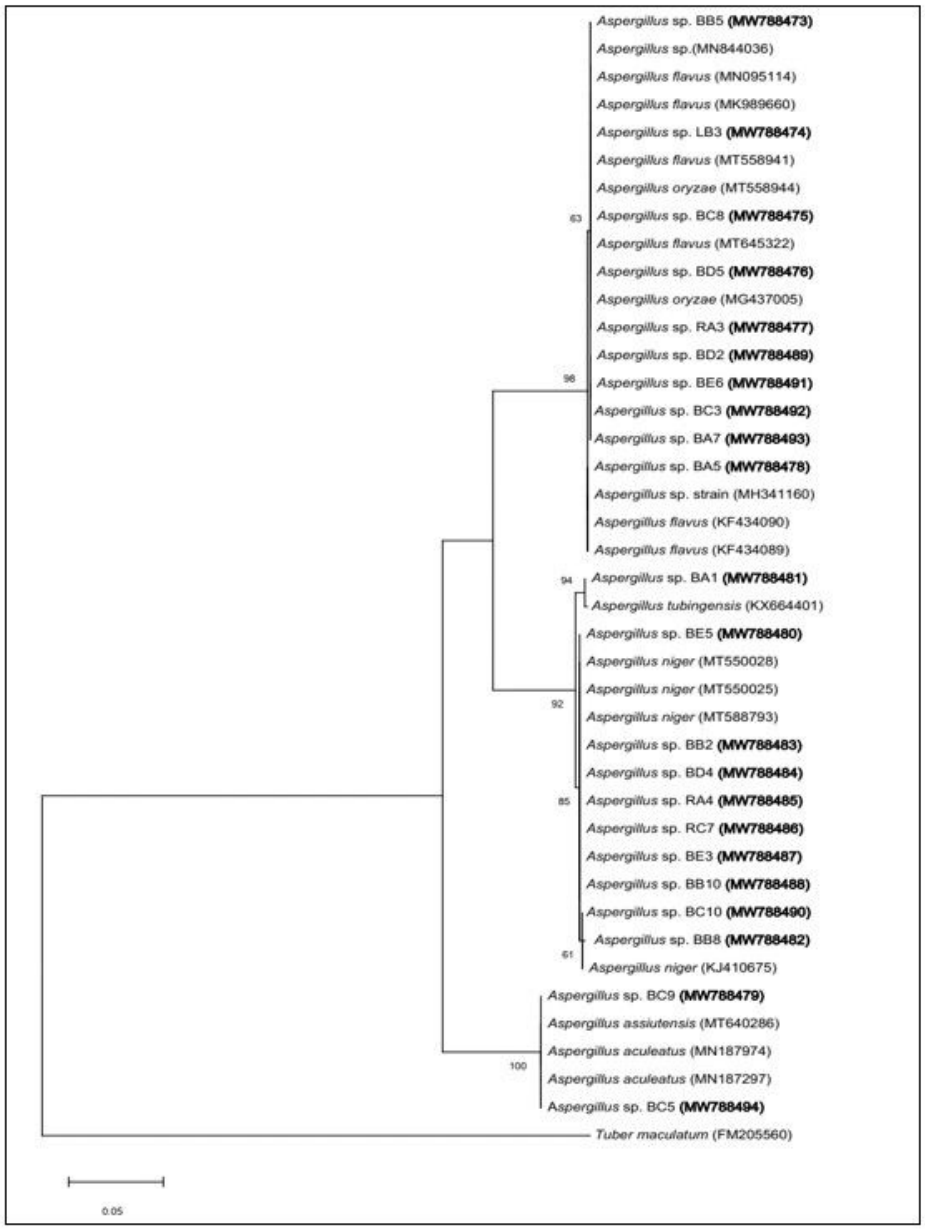

Figure 1

Evolutionary relationships between partial ITS gene sequences of the isolates and some closest known fungal species. *Tuber maculatum* (Accession No.FM205560) was used to root the phylogenetic tree. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Bar, 0.05 substitutions per nucleotide position