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Orignal Article

Isolation of Antimicrobial Producing Actinomycetes from Indigenous Microhabitats

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ABSTRACT

The number of microorganisms expressing antimicrobial resistance phenotypes, the global spread, and the growing range of antimicrobial resistance capabilities of individual microbial species have become unprecedented. The mechanism of antimicrobial resistance for most microbial pathogens, especially many pathogenic fungi, and bacteria include the production of enzymes that attack the active compounds of drugs, the expression of efflux pumps, the by-pass of metabolic pathways, and the modification of drug target sites. Unusual microhabitats in different locations present opportunities for the bio-prospecting of actinomycetes with high antimicrobial producing potentials. **Objective:** To isolate and characterize antagonistic actinomycetes from indigenous microhabitats. **Methods:** A total of 113 actinomycetes from specific indigenous microhabitats were isolated and screened for their antimicrobial activity against selected test organisms. The earthworm castings, forest litter, and domestic dumpsite sites, respectively, accounted for approximately 24.78%, 19.47%, and 18.5% of the total number of isolates recovered. The preliminary antagonism assay using the perpendicular streak method yielded four isolates that showed high antimicrobial activity and broad-spectrum activity against test bacteria or fungi. **Results:** These isolates showed excellent growth and sporulation on milk agar and starch casein agar, and the micro-morphological characterization of the isolates revealed the presence of spore-bearing aerial hyphae. Bioactive extracts from DS15 and EC5, at 125 mg/mL, had respective zones of inhibition measuring 20.7 ± 0.6 and 33.0 ± 1.0 against multidrug-resistant *Escherichia coli* (clinical isolate) and *Salmonella typhi* (ATCC 2923) respectively. Bioactive extracts from EC1 and EC9, at 125 mg/mL, show respective zones of inhibition measuring 18.7 ± 1.2 and 16.7 ± 1.2 against *Candida albicans* (ATCC 10231) and *C. glabrata* (ATCC 22018) respectively. **Conclusions:** This study reinforces the evidence that unique and unusual microhabitats, in different geographical locations, present an excellent opportunity for the bioprospecting of actinomycetes with high antimicrobial producing potentials. Furthermore, it shows that the indigenous antagonistic actinomycete isolates exhibited potent antimicrobial activity against test bacteria and fungi employed in the in-vitro antimicrobial assays.

INTRODUCTION

The number of microorganisms expressing antimicrobial resistance phenotypes, the global spread, and the growing range of antimicrobial resistance capabilities of individual microbial species has become unprecedented [1]. The mechanism of antimicrobial resistance for most microbial pathogens, especially many pathogenic fungi, and bacteria include the production of enzymes that attack the active compounds of drugs, the expression of efflux pumps, the by-pass of metabolic pathways, and the modification of drug target sites [2]. These processes have rendered many first-line drugs impotent and negatively impacted the quality of life of many. Presently, about 700,000 people die annually from drug-resistant diseases [3], and the World Bank predicts that antimicrobial resistance could pull 24 million people into extreme poverty by 2030 and cause a fall of 1.1–3.8% in global GDP by 2050 [4]. In the drive to add to humanity's depleting antimicrobial armory, researchers have turned to the actinomycetes. From antimicrobials,

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and anti-tumor agents, to enzymes and immunosuppressants, they are responsible for over 45 percent of all bioactive compounds obtained from microbes [5]. Some actinomycete strains possess biosynthetic gene clusters that encompass more than 16 percent of their entire coding capacity— producing 35 to 53 distinct secondary metabolites [6]. Since the days of the pioneering works of Selman Waksman, the bioprospecting of the actinomycetes has seen scientists traverse unusual terrains, geographical locations, and habitats in search of bioactive secondary metabolites. Antagonistic actinomycetes have been investigated in soil samples from varying altitudes ranging from 1500 to 4380 meters above sea level across the mountainous terrains of Nepal [7]. Other studies have researched extensively on actinomycetes from arid [8] and marine habitats [9]. More peculiar studies have looked at actinomycetes symbionts within micro-habitats such as: the rhizosphere of leguminous plants [10]; the gastrointestinal tracts of ruminants [11]; and within venomous cone-snails [12]. They are known to exist in various synergistic relationships with plants, lesser invertebrates, and insects. Expectedly, the exploration of actinomycetes, within microhabitats, has emerged as a new frontier for the prospecting of antagonistic strains [12].Within this context, this study aims to isolate and characterize antagonistic actinomycetes from indigenous microhabitats. Although similar studies have explored some of these sites, there are limited studies on antagonistic actinomycetes isolated from samples collected in the study area.

METHODS

Umuahia-North local government area is one of the two local government areas that constitute Umuahia, the capital of Abia State in Nigeria. It has an area of 245 square kilometers [13], and is located in the lowland rainforest zone of Nigeria within the geographical coordinates, latitude 5.5262700 and longitude 7.4895900 (decimal degrees)[13]. It has an average rainfall of 2238 mm per year that is distributed over a seven-month rainy season [14]. Umuahia North has an estimated 19, 689.09 hectares of vegetal cover with an average daily temperature of 31 o C, and relative humidity of 67% [13]. The Samples were sourced from rhizospheric soil, domestic waste disposal site soil, forest litter soil, earthworm castings, anthill soil, mosses, and dry tree barks. Fifty grams of each sample were collected into clean polyethylene bags and transported to the laboratory for analysis. The soil samples were air-dried and sieved with a 2mm mesh sieve to remove stones and debris. Subsequently, one gram from each soil sample was mixed with 0.1 g of CaCO3 and incubated at 26 0C for 7 days in an atmosphere saturated with moisture [15]. For the tree bark samples and mosses, 1 g of the pulverized samples was suspended in 10 mL sterile normal physiological saline, and then vigorously mixed [16].

Isolation and presumptive identification of actinomycetes colonies: 0.1 mL from each of the dilution factors 10-3, 10-4, and 10-5 was inoculated unto starch casein agar (SCA), yeast extract-malt extract agar (ISP-2) and inorganic salts-starch agar (ISP-4) media using the spread plate technique [17]. Prior to inoculation, those media were impregnated with rifampicin (2.5 micrograms per mL) and nystatin (50 micrograms per mL) to forestall contaminants. The inoculated plates were aerobically incubated at 250 C for a minimum of 7 days and observed intermittently for growth [18]. Tentative actinomycetes colonies were presumptively identified as microcolonies with a leathery texture, adhering to the agar.

Test organisms: Gram-positive bacteria: Staphylococcus aureus (ATCC 25923); Bacillus cereus (IFO 13804); and multidrug-resistant S. aureus (clinical isolate). Gram negative bacteria: E. coli (JCM 20135); Salmonella typhi (ATCC 2923); Pseudomonas aeruginosa (ATCC 25783); multi drug resistant P. aeruginosa (clinical isolate); Klebsiella pneumonia (clinical isolate); and multi drug resistant E. coli (clinical isolate). Yeast strains: Candida albicans (ATCC 10231) and C. glabrata (ATCC 22018).

Preliminary screening of actinomycetes for antimicrobial activity: Antagonism assay of the actinomycete isolates against selected test bacteria and fungi were done using the perpendicular streak method on Mueller-Hinton agar and SDA for test bacteria and fungi respectively [5]. The zones of inhibition were measured and those isolates that exhibited high antimicrobial activity were chosen for subsequent characterization, and crude bioactive substance production [5]. Performance was judged based on the following criteria: broad-spectrum activity and high antimicrobial activity. The best two (2) performers from the antibacterial and antifungal assay respectively were selected for further investigation.

Morphological characterization: The morphological characterization was done using ISP-2, ISP-4, SCA, SDA, and milk agar. These media were inoculated with 0.05 mL of inoculum and streaked following the method described by Shirling and Gottlieb [19]. The plates were incubated in the dark at ambient room temperature (28 o C) and observed at the end of 7, 14, and 21 days. The micro-morphological characteristics of the spore-bearing aerial hyphae, the mass color of mature, sporulating aerial surface growth, and color of substrate mycelium (reverse color) were subsequently determined [19].

Physiological characterization: The physiological characterization of the selected isolates involved the determination of melanoid pigments production and

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carbon utilization patterns. This was determined according to the method described by Shirling and Gottlieb [19]. Biochemical characterization: The biochemical profile of the selected isolates was determined by through several biochemical tests, and these include: Gram's staining, starch hydrolysis, gelatin hydrolysis, urea hydrolysis, casein hydrolysis, H2S production test, citrate utilization test, sodium chloride tolerance test, and catalase production test [5].

Extraction of antimicrobial compounds: The selected isolates showing high antimicrobial activities from the preliminary screening were grown as submerged cultures in 250 mL flasks containing 50 mL of ISP-2 broth media. The flasks were then inoculated with 2 mL suspension of the isolates and kept in a thermostat rotary shaker for 7 days at 28oC (at 250 rpm). The cell-free supernatant was obtained following filtration of the culture broth with the aid of a cheesecloth [18,20]. Equal volumes (1:1 v/v) of ethyl acetate were added to the resultant supernatant and shaken vigorously and allowed to stand for one hour. The solvent phase was separated from the aqueous phase by using a 500 mL separating funnel and then subjected to a Rota-vapor apparatus (at 60oC and at 100 r/mins) in order to obtain the crude bioactive extract. The crude extract from the extraction was subsequently recuperated in 20% dimethyl sulfoxide [5].

Antimicrobial activity: Standard diffusion antimicrobial sensitivity assays were employed to assess the antimicrobial activity of the crude bioactive extracts [21]. Agar well diffusion method was used to determine to mean zones of inhibition while standard broth micro-dilution testing was employed in the determination of the minimum inhibitory concentration. For the agar, well diffusion method, the zones of inhibition were measured and the mean with a standard deviation of the triplicate experiment was subsequently calculated.

Long-term preservation of actinomycete isolates: The mycelium of high-performing actinomycete isolates was stored according to the 'Basic Protocol 4' method described by Shepherd et al. [22].

Statistical analysis: The mean and standard deviation of triplicate experiments were computed using the Statistical Package for Social Science software (SPSS) version 16. Graphic data visualization software was used to build figures.

RESULTS

Isolation and presumptive identification of actinomycetes colonies: Using the selective media and cultural conditions described previously, a total number of 113 presumptive actinomycetes isolates were recovered from 21 samples collected from the selected sites: rizopheric soil 11.5%; mosses 8.9%; tree barks 7.1%; domestic dumpsites 18.6%; decaying vegetation 19.5%; earthworm castings 24.8%; and termite hill 9.73% (Figure 1).

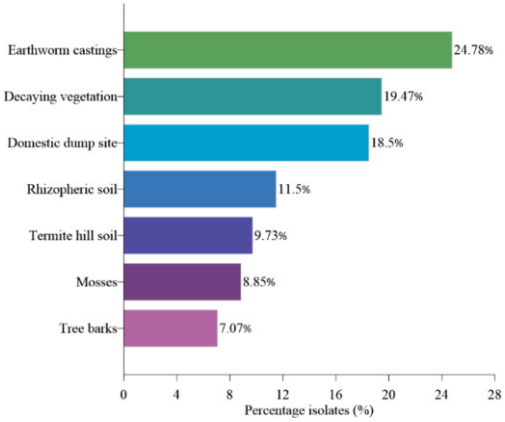

Figure 1: Distribution of actinomycetes population isolated from the various microhabitats

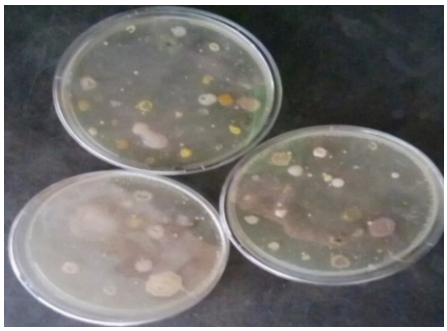

Figure 2: Plate 1-Primary isolation of actinomycetes on Starch Casien Agar(SCA)

Preliminary screening of actinomycetes for antimicrobial activity: From the screening, 27 isolates exhibited antagonism against one or more of the bacterial strains employed in the assay. However, only 7 isolates (DV2, DS31, DS15, DSC4, EC5, TB11 and EC7) among the 27 positive isolates, showed a relatively high antibacterial activity against the bacterial test strains. In contrast, most of the isolates screened for antifungal activity showed no inhibitory activity against the selected fungal test strains except isolates EC9, EC1 and TH2, which showed visible activity. Isolates that showed good performance were chosen for subsequent characterization and crude bioactive substance production.

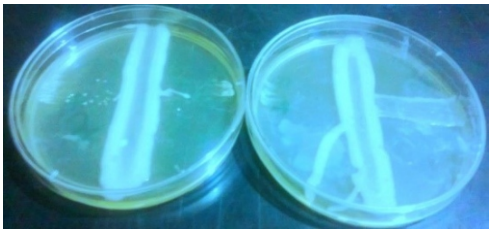

Figure 3: Plate 2-Single streak across the middle of antagonistic

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isolates EC1 (right) and EC9 (left) showing inhibition (arrow) against C. albicans and C. glabrata in each plate, respectively

Morphological characterization of selected isolates: The spore-bearing aerial hyphae of the four best performing isolates reveal that, excluding isolate DS15 (verticillate), they all fall under the category "simple". In addition, the presence of spore-bearing aerial hyphae among all the isolates indicates that they are of the Streptomyces genus. The result of the macro-morphological characteristics of the selected isolates reveal that all selected isolates show excellent sporulation on Milk agar and Starch Casein Agar while varying growth patterns were observed on the other growth media used. Only isolate EC5 exhibited the production of diffusible pigments other than melanin. The pigment showed a characteristic yellow-brown color with a strongly visible red tint. Physiological characterization of the selected isolates: The results for the physiological characterization of the selected isolates reveal that they all showed a strong positive utilization of galactose and mannose as a source of carbon. However, the utilization patterns shown by the selected isolates for D-sorbitol reveal that it was relatively the least utilized among the carbon sources. Also, the production of melanin pigment was absent among the selected isolates with an exception of isolate EC5 which consistently produced melanin pigment on various growth media used (Table 2).

Variables	Isolates			
Test performed	**DS15**	**EC5**	**EC1**	**EC9**
Melanin production	-	+	-	-
No carbon source (-ve)	-	-	-	-
D-glucose (+ve)	++	++	++	++
L-arabinose	+	+	++	+
Sucrose	+	+	+	+
I-inositol	+	+	-	+
D-fructose	++	+	+	++
Galactose	++	++	++	++
D-sorbitol	+	-	-	+
Maltose	+	+	+	+
Mannose	++	++	++	++
D-mannitol	+	+	+	+

++: strong positive utilization; **+:** positive utilization; **-:** negative utilization

Table 2: Physiological characteristics of the selected isolates

Biochemical characterization of the selected isolates: Biochemical characterization results indicate that in addition to the production of catalase, all of the selected isolates were positive for the ability to hydrolyze starch, gelatin, and casein. The results also show that most of the selected isolates were negative for the ability to produce urease and citrase enzymes (Table 3).

	Isolates			
Test performed	**DS15**	**EC5**	**EC1**	**EC9**
Gram's reaction	+	+	+	+
Gelatin hydrolysis	+	+	+	+
Starch hydrolysis	+	+	+	+
Casein hydrolysis	+	+	+	+
Urea hydrolysis	-	-	-	+
Citrate hydrolysis	-	-	-	-
NaCl tolerance	4-7%	4%	4%	4%
H_2S production	+	+	-	-
Catalase production	+	+	+	+

+: positive; **-:** negative

Table 3: Biochemical characteristics of the selected isolates

Antimicrobial activity

Agar well diffusion assay: As shown in Table 4, the two crude extracts from DS15 and EC5, at their respective concentrations, showed varying degrees of activity against some of the non-multidrug-resistant bacteria tested but showed no activity against multidrug-resistant (MDR) K. pneumoniae (clinical isolate), MDR P. aeruginsosa (clinical isolate), and P. aeruginosa (ATCC 25783). However, MDR S.aureus (clinical isolate) and MDR E.coli were found to be somewhat susceptible to the crude extract from isolate DS15. Also, the zones of inhibition of the selected test fungi at different concentrations of the crude extract from EC9 and EC1 isolate were measured. The results obtained reveal that the two crude extracts performed favorably, and showed similar activities against Candida albicans (ATCC 10231) and C. glabrata (ATCC 22018).

Extract Conc./ Test microorganism	125 mg/mL		62.5 mg/mL		31.25 mg/mL		15.63 mg/mL	
	DS15	EC5	DS15	EC5	DS15	EC5	DS15	EC5
E. coli	33.7±4.7	20.7 ±3.1	20.3 ±1.5	10.0 ±1.0	13.7 ±1.5	3.7±0.6	3.3±1.2	1.3±1.2
B.cereus	33.0 ±2.6	32.7 ±1.5	25.7 ±1.5	17.3 ±3.1	14.3 ±1.2	9.0±2.6	6.7±1.5	3.0±1.0
S. aureus	31.0 ±1.0	20.7 ±1.5	21.0 ±1.0	10.0 ±1.0	12.7 ±2.5	4.3±0.6	5.0±1.0	1.3±0.6
†*S. aureus*	19.7 ±0.6	-	14.7 ±2.1	-	7.3 ± 2.1	-	2.7±1.5	-
†*K. pneumoniae*	-	-	-	-	-	-	-	-
†*P. aeroginosa*	-	-	-	-	-	-	-	-
†*E. coli*	20.7 ±0.6	-	13.3 ±1.5	-	7.0 ± 1.0	-	2.7±0.6	-
S. typhi	-	33.0 ±1.0	-	19.0 ±2.6	-	8.7±0.6	-	3.3±0.6
P. aeroginosa	-	-	-	-	-	-	-	-
	EC9	EC1	EC9	EC1	EC9	EC1	EC9	EC1
C. albicans	18.7±1.2	23.0 ±1.0	13.3 ±2.1	13.3 ± 0.6	8.7±0.6	8.3±1.2	2.7±1.2	4.3 ±0.6
C. glabrata	16.7±1.2	20.3 ±1.5	8.7 ± 1.5	11.3 ± 0.6	5.3±0.6	7.3±0.6	1.3±0.6	2.3 ±1.2

†Multi drug resistant

Table 4: The mean zones of inhibition (mm) of selected test microorganisms at different concentrations of the crude bioactive extracts from selected isolates

Micro Dilution Minimum Inhibitory Concentration Assay: As shown in Table 5, The bioactive extract from isolate DS15 had MIC values of 250 µg/mL, 250 µg/mL, 500 µg/mL, and 500 µg/mL against E. coli (JCM 20135), S. aureus (ATCC 25783), MDR S. aureus (clinical isolate) and MDR E. coli

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(clinical isolate) respectively. The MIC values (µg/mL) of the bioactive extract from isolate EC5 were 250 µg/mL, 250 µg/mL and 500 µg/mL against E. coli (JCM 20135), S. aureus (ATCC 25783) and S. typhi (ATCC 2923) respectively. The bioactive extract from isolate EC9 had MIC's of 125 µg/mL and 250 µg/mL against Candida albicans (ATCC 10231) and C. glabrata (ATCC 22018) respectively while the bioactive extract from EC1 had MIC's of 125 µg/mL and 250 µg/mL against the same test fungi.

Test microorganism	Isolates		Comparison
	Ds15	Ec5	CPR
E. coli	>250	>250	>2
S. aureus	>250	>250	>0.5
S. typhi†	Nd	>500	>0.5
S. aureus†	>500	Nd	>0.5
E. coli	>500	Nd	>0.25
	Ec9	Ec1	KCZ
C. albicans	>125	>125	>6.25
C. glabrata	>250	>125	>12.5

Table 5: MIC (µg/mL) of the bioactive extracts from the selected isolates

CPR: Ciprofloxacin **KCZ:** Ketoconazole **Nd:** Not determined

†Multi drug-resistant

DISCUSSION

In the present study, a total of 113 presumptive actinomycete isolates were got from soil samples that were randomly collected from plant rhizophere, mosses, tree barks, domestic dumpsites, forest filters, earthworm castings, and termite hill. The cumulative percentage of isolates recovered from earthworm castings, decaying vegetation, and domestic dumpsite sites represent more than half (62.75%) of the total number of isolates recovered. Similar studies suggest that the high rate of recovery from such sites is due to the high humus content of soil from those niches [23]. Among these aforementioned microhabitats, the earthworm castings (24.78%) represent an important prospect for the isolation of novel actinomycetes as the literature suggests that casting activity leads to nutrition and microbial enrichment [24]. Although the isolation of actinomycetes from various sites can be done with ease, the frequency of isolating an antibiotic-producing strain is relatively low [25]. The screening for antagonistic strains using the single streak technique revealed that 23.89% of the isolates showed antagonism against one or more of the bacterial strains used in the assay. Low frequencies of isolating antibiotic-producing actinomycetes have also been reported by other studies [5,17]. Following the screening for isolates against selected yeast strains, only 3% of the total number of isolates returned positive. This percentage is similar to those reported by other investigators (5-9%) [16,26]. This outcome may reflect the intrinsic properties of the fungi which makes it resistant to most bioactive compounds produced by actinomycetes [27]. The micro-morphological characteristic of the spore bearing aerial hyphae of actinomycetes is an important criterion in the characterization of an actinomycete isolate [19]. The presence of spore-bearing aerial hyphae indicates that a particular isolate is of the Streptomyces genus [19]. The results reveal that isolate EC5, EC1, and EC9 showed spore-bearing hyphae that ranged from 'Rectus' to 'Spira.' Investigators who worked on soil from Antarctica, report that most of the isolated strains ranged from 'Rectus' to 'Flexibilis' [28]. The isolate DS15 was peculiar from the rest in that it had a 'Monoverticillus' to 'Spira' spore-bearing aerial hyphae morphology. Other criteria of the actinomycetes that have been used extensively in its taxonomy are the color of the sporulating aerial mycelia and substrate mycelia, sporulation, and production of pigments [19]. The color of the sporulating aerial hyphae of the chosen isolates ranged from white to grey. These observations are similar to those reported by studies from Ethiopia (grey to white to chocolate) [5], Iran (grey to white) [29], and Bengal (grey to white) [25] respectively. Those investigators also report that the color of the substrate mycelia of their respective isolates ranged from yellow to brown. These observations are consistent with those made in the present study in that most of our isolates have reverse colors that ranged from yellow to brown except isolate EC5, which shows a characteristic yellow to brown color with a strongly visible red tint. The production of pigments was only peculiar to the isolate EC5 while the other three isolates were found to be lacking in this attribute. The ability of the spores of some species of actinomycetes to survive in hostile environments may be increased by the presence of pigments and aroma in the spores of those species, which may stimulate cell development and secondary metabolite production [30]. The growth and maintenance of Streptomyces on agar media are very important for several reasons, such as the production of secondary metabolites; and various genetic manipulation protocols, which include electroporation and conjugal transfer of DNA [22]. The ability of actinomycetes to produce spores on solid media is also used as an indicator of growth efficiency [22]. Our findings show that the chosen isolates showed excellent growth on starch casein agar and milk agar, but showed varying growth patterns on the other media used. Although the exuberant growth of actinomycetes on starch casein agar has been well documented [5], the elaborate growth on milk agar is however novel in some sense. The lack of literature on the excellent sporulation of actinomycetes on milk agar may be due to the fact that the agar is readily prone to proteolysis, and unsuitable for long term storage of grown cultures. This study reveals that the chosen isolates showed a strong

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positive utilization of galactose and mannose as a source of carbon while D-sorbitol was seen as the least utilized among the carbon sources assayed. Investigators have reported a similar pattern for the utilization of galactose and manose [31]. Also, the poor utilization of D-sorbitol as a carbon source has been reported by a study in India [25]. The actinomycetes have a large genome consisting of a plethora of genes encoding mediators of various catabolic and anabolic pathways [32]. This innate ability of the actinomycetes has been explored and utilized for various industrial and pharmaceutical purposes [17]. Our isolates were found to be positive for the ability to hydrolyze starch, gelatin and casein and for the ability to produce catalase. Following the growth of the isolates in 1SP-2 broth the bioactive crude substances were extracted using ethyl acetate. It has been reported that the use of 1SP-2 medium for submerged fermentation of antibiotic producing isolates showed very good results [20]. Ethyl acetate was chosen because of its non-toxic nature and because it does not interfere with susceptibility assays [33]. Different concentrations of the respective bioactive crude substances were used for well in agar diffusion susceptibility assays, and this reflects the fact that several antibiotics show a concentration dependent activity e.g. the aminoglycosides and quinolonesm [34]. Antibacterial assay of the bioactive extracts from DS15 and EC5 reveals that while the two crude extracts at respective concentrations showed varying degrees of activity against some of the bacteria tested, neither of the two crude extracts at those concentrations showed activity against multi-drug resistant K. pneumoniae (clinical isolate), MDR P. aeruginsosa (clinical isolate) and P. aeruginosa (ATCC 25783). This observation may be due to the presence of resistance and/or because more than 60% of known antibiotics produced by actinomycetes have been found to be active against gram-positive bacteria only [34]. Interestingly, the bioactive extracts from DS15 and EC5 showed activity against E. coli and S. typhi respectively. he MIC values (µg/mL) of the bioactive crude extract from DS15 against Gram-negative bacteria were 250 (µg/mL) and 500 (µg/mL) for E. coli (JCM 20135) and MDR E. coli (clinical isolate) respectively. Studies have reported MIC values that ranged from 128 µg/mL [35] to 250 µg/mL [36] against some strains of E. coli. The MIC values of the extract against the MDR E. coli strain used was expectedly high and this could be due to the multi-drug resistance profile of the clinical isolate. The MIC values of the extract (DS15) against Gram-positive bacteria were 250 µg/mL and 500 µg/mL for S. aureus (ATCC 25783) and MDR S. aureus (clinical isolate) respectively. The MIC value of our extract against S. aureus (ATCC 25783) was much lower than what was reported by studies in India (500 µg/mL) [24] and Ethiopia (3.30 mg/mL) [5], respectively for the bacterial species. We could also attribute the high MIC value of the extract against the MDR S. aureus (clinical isolate) strain used to the multi-drug resistance profile of the clinical isolate. The MIC values (µg/mL) of the bioactive crude extract from isolate EC5 against Gram-negative bacteria were 250 (µg/mL) and 500 (µg/mL) for E. coli (JCM 20135) and S. typhi (ATCC 2923) respectively. While for the Gram-positive bacterium S. aureus (ATCC 25783), an MIC value of 250 (µg/mL) was recorded. These values varied from what has been reported in other studies against the respective test bacteria [23,35]. The results for the antifungal assay of the bioactive extracts from EC9 and EC1 reveal that the two crude extracts at respective concentrations showed comparatively similar activities against Candida albicans (ATCC 10231) and C. glabrata (ATCC 22018). The bioactive extract from isolate EC9 had MIC values of 125 µg/mL and 250 µg/mL against Candida albicans (ATCC 10231) and C. glabrata (ATCC 22018) respectively while the bioactive extract from EC1 had MIC values of 125 µg/mL and 125 µg/mL against the same test fungi. Varying results against C. albicans test strains have been reported by similar studies in Thailand (15.87 µg/mL) [37], Algeria (50 µg/mL) [20], and India (1000 µg/mL) [24]. These MIC values varies markedly from our results and show that different antibiotic-producing actinomycetes isolated from different geographical sites exhibit distinct antimicrobial activity even against the same test microorganism. With an MIC value range of 125 µg/mL to 250 µg/mL, the bioactive extracts reported in the present study showed a comparatively better activity index against C. glabrata than that reported by a study that investigated antagonistic actinomycetes from ants (250 to 1000 µg/mL) [38].

DISCUSSION

This study reinforces the evidence that unique microhabitats present an excellent opportunity for the bio-prospecting of actinomycetes with high antimicrobial producing potentials. The earthworm castings, decaying vegetation, and domestic dumpsite site accounted for approximately 24.78%, 19.47%, and 18.5% respectively of the total number of isolates recovered. Earthworm castings, in particular, show a great promise for the future of antimicrobial bio-prospecting as three out of the four best antagonistic actinomycete isolates were recovered from this microhabitat. Furthermore, it shows that the indigenous antagonistic actinomycete isolates exhibited potent antimicrobial activity against some of the selected test bacteria and fungi employed in the in-vitro antimicrobial assays. Future studies will benefit from focusing on the purification and elucidation of the structure of the active compound, and in-vivo cytotoxicity

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assays. Also, the genomes of these isolates will be explored for the presence of cryptic antibiotic pathways and the information can be updated to ongoing actinomycetes genome projects.

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