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Antimicrobial Activity of *Actinomycetes* Isolates from Different Soil Samples in Ikere-Ekiti, Ekiti State, Nigeria

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ABSTRACT

Actinomycetes are a group of Gram-positive, facultatively anaerobic soil bacteria known for their ability to produce bioactive substances. This study analysed the antimicrobial potential of *Actinomycetes* isolates from soil samples collected from various locations within the University campus in Ikere-Ekiti. Samples were serially diluted and cultured on Starch Casein Agar supplemented with Fluconazole and Nalidixic acid antibiotics. Isolates were characterised morphologically and biochemically, followed by primary and secondary screenings for antimicrobial activity using perpendicular-streak and agar-well diffusion techniques. Colony morphology exhibits variation in size, shape, elevation, edge, surface texture, consistency, and pigmentation. Mean total *Actinomycetes* count ranged from 0.813 to 0.881 log cfu/mL at dilution 10^{-2} and 0.378 to 0.602 log cfu/mL at dilution $10^{-3.}$ All isolates were Gram-positive, and biochemical tests revealed that approximately 15.4% of the isolates were catalase-positive, while about 33.3% demonstrated indole positivity. The recovered 30 *Actinomycetes* isolates were screened against eight test organisms: *Escherichia coli*, *Klebsiella* sp., *Pseudomonas* sp., *Proteus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Aspergillus flavus* and *Aspergillus niger*. Isolates SB3[1] and DB3 were active against all the test bacteria during primary screening, while isolate SB3[1] showed activity against only four test bacteria during secondary screening. Furthermore, nine out of 27 isolates predominantly exhibited antifungal activity against *A. flavus* and *A. niger* during both primary and secondary screening. These findings show the *Actinomycetes* isolates are a promising source of antimicrobial compounds.

Keywords: *Actinomycetes*, Antibacterial activity, Antifungal activity, Bioactive compounds

Introduction

Actinomycetes are a diverse group of Gram-positive, filamentous bacteria that are widely distributed across terrestrial and marine ecosystems. These microorganisms have attracted significant attention due to their remarkable ability to produce a broad spectrum of secondary metabolites with diverse biological activities. [1] Of particular interest is their ability to make compounds with antimicrobial and larvicidal properties, making them not only key players in the discovery of novel antibiotics but also in establishing and regulating environment-friendly and sustainable natural pest control frameworks. Notable *Actinomycetes* include species from the *Streptomyces* genus, renowned for producing secondary metabolites, including well-known antibiotics such as streptomycin, tetracycline, and erythromycin, which have had profound impacts on human health. [1] Beyond their antibiotic properties, *Actinomycetes* have gained prominence as a promising source of bioactive compounds for combating vector-borne diseases, particularly those targeting mosquito larvae.

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The increasing global threat of antimicrobial resistance (AMR) is a call for a renewed effort to develop new and effective antibiotics. Antimicrobial resistance (AMR) is one of the most significant global health challenges of the 21st century.[2] The rise of resistant bacterial strains has rendered many conventional antibiotics ineffective, leading to increased morbidity, mortality, and healthcare costs. The search for novel antimicrobial agents has therefore become critical in combating the menace of AMR. *Actinomycetes*, a group of soil-dwelling microorganisms, have long been recognized as a valuable source of bioactive compounds, including antibiotics, due to their ability to produce a wide range of bioactive secondary metabolites. These microorganisms thrive in different ecological environments, and their metabolic products often exhibit potent antibacterial, antifungal, and antiviral properties.[3] The ability of *Actinomycetes* to produce antibiotics and other bioactive substances is often influenced by various nascent environmental factors, making the collection of soil samples from different geographic regions important for discovering novel compounds.[4]

Given the predominant agrarian practice in Ikere-Ekiti, western Nigeria, the soil offers a rich and diverse ecological setting. This area, characterized by its varied vegetation and agricultural activities, is an ideal environment for thriving *Actinomycetes* populations. However, there is limited research on the antimicrobial potential of *Actinomycetes* from this region, and studies in the microbial diversity of Ikere-Ekiti are sparse. Therefore, exploring the *Actinomycetes* populations in this ecosystem could yield novel strains with potential antibacterial secondary metabolites for addressing the growing concern of antibiotic resistance. [5] Therefore, discovering new *Actinomycetes* with antibacterial or antifungal properties could be a critical step in the development of next-generation antibiotics[6]. This discovery will address the global challenge of antimicrobial resistance while also providing new insights into the microbial diversity of the region. Here, we investigated the antimicrobial activity profiles of *Actinomycetes*

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isolated from stream, decomposed organic matter, and loamy soil samples in Ikere, Ekiti State, Nigeria using standard agar well diffusion techniques.

Materials and Methods

Study Area

Soil samples from dung, stream sediment and loamy soil were collected from different locations of Bamidele Olumilua University of Education, Science and Technology, Ikere-Ekiti (BOUESTI), Ekiti State (Figure 1). Soil Sample Collection: Fifty-four (54) samples from stream sediment, loamy soil, and dung soil were collected within the depth of 10–12.5 cm and labelled with the alphabets: A, B, C, D, and E. Similarly, the isolates from each respective sample were labelled according to their sources: stream: SA, SB, SC, SD, SE, loamy: LA, LB, LC, LD LE, and decomposed soils: DA, DB, DC, DD, DE. All samples were collected in sterile plain sample bottles and taken to the laboratory for analysis.

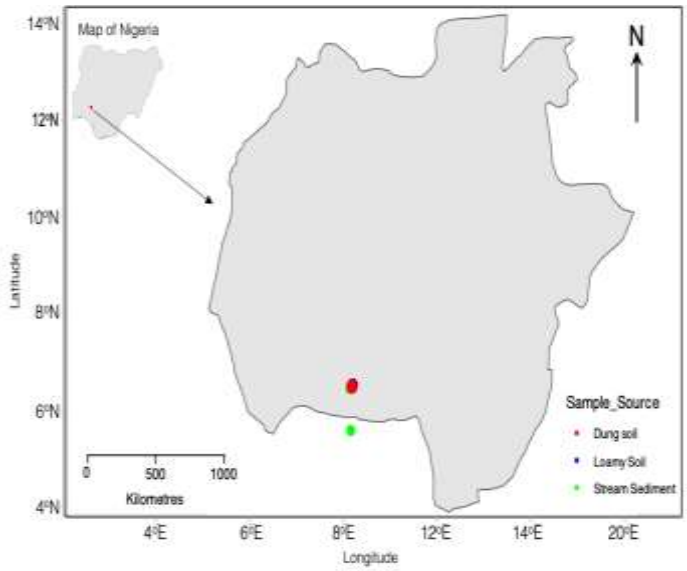

Figure 1: A map plot of Ekiti State, showing sampling locations in the Bamidele Olumilua University of Education, Science & Technology, Ikere (BOUESTI) metropolitan area, was made using R Statistical Computing Software. The point plots display the latitude and longitude coordinates of the sampling areas, coloured by source. The collection sites for the loamy and dung soil samples slightly overlap.

Isolation of Samples

Samples collected were cultured on starch casein agar (RDM-SMPA-01) (ReadyMED, India), supplemented with Fluconazole (0.5 mg/mL of media) (SMOTEC Pharmaceuticals) and Nalidixic acid (1 mg/mL of media) (GLITZ Pharma), using the pour plate technique. The plates were incubated at 28°C for 7 days. The isolates were counted and subcultured on starch-casein agar and then stored on agar slants for further studies.

Identification and Characterisation of Actinomycetes

The identification and characterisation of *Actinomycetes* isolates were accomplished using cultural characteristics, the Gram staining technique, and biochemical tests. For presumptive identification of the isolates, the morphology of the isolates was compared to the *Actinomycetes* morphology listed in Bergey's Manual of Systematic Bacteriology. [7]

Test organisms

Bacterial test organisms already identified were collected from the Microbiology Research Laboratory at Federal Polytechnic, Ado-Ekiti, Nigeria. These included *Escherichia coli*, *Klebsiella* sp., *Pseudomonas* sp., *Proteus* sp., *Salmonella* sp., and *Staphylococcus* sp. At the same time, the fungal test organisms were *Aspergilus niger* and *Aspergilus flavus.*

Primary Screening of Isolated Actinomycetes

The primary screening of the inhibitory activities of Actinomycetes isolates against test bacterial and fungal species was conducted using the previously described perpendicular streak method. [8, 9] Eight to nine isolates were streaked across the middle of a Mueller-Hinton agar plate and incubated at 28 °C until an adherent vertical colony line appeared. Following the incubation, the test organisms—*Escherichia coli*, *Klebsiella* sp., *Pseudomonas* sp., *Proteus* sp., *Salmonella* sp, *Staphylococcus* sp, *Aspergillus niger* and *Aspergillus flavus*—were individually streaked in a perpendicular direction to the *Actinomycetes* colonies and then incubated for one day at 37 °C, and five days at room temperature for bacterial and fungal test organisms, respectively. After incubation, plates were inspected for inhibitory activities of the *Actinomycetes* isolates on the test organisms.

Fermentation for the production of Actinomycetes bioactive metabolites

Potent isolates from the primary screening were identified and subjected to secondary screening, following the procedure employed by Ibnouf et al. [10]. Starch-casein broth was prepared in a conical flask for the cultivation of metabolite-producing potent isolates. Each clone of identified *Actinomycetes* that exhibited the highest activity against test organisms during primary screening was added to the broth, and the conical flasks were incubated at 28 °C for 8-12 days.

Extraction of Metabolites from Actinomycetes for Secondary Antimicrobial Screening

Crude extraction of metabolites from the broth of *Actinomycetes* was performed using the centrifugation technique as described by Ibnouf et al. [10] After 8-12 days of incubation, the broth culture was centrifuged using a high-speed refrigerated centrifuge (TGL16A) (Kaida Lab) at 4000 rpm for 30 minutes. The supernatant was decanted and subjected to a second round of centrifugation at 4000 rpm for 30 minutes. Followed by decantation of the supernatant and storage at 4 °C for further use.

Secondary Screening of Actinomycetes for Antimicrobial Activities

The Agar well diffusion technique was used for the secondary screening of metabolites extracted from *Actinomycetes*, following the procedure described by Sivarajan et al. [11]. A 24-hour-old culture of the test bacteria was inoculated on Mueller-Hinton agar using the spread technique, after achieving the 0.5 McFarland standard. Five (5) wells were bored using a sterile cork borer, and 0.4 microlitres of the crude extracts were dispensed into the wells, and sterile distilled water was used as a control. The plates were incubated for one day at 37 °C and for five days at room temperature for bacterial and fungal test organisms, respectively. Afterwards, the plates were inspected, and the diameter of the inhibition zones surrounding each well was measured in millimetres (mm).

Data Analysis

All data analysis and visualisations were performed using R Statistical Computing Software, Version 4.5.0.

Results and Discussion

The colonial appearance of *Actinomycetes* on Starch Casein Agar is shown in Plate 1. The mean colony counts for each sample are presented in Table 1, which reveals that loamy soil samples had the fewest colonies of *Actinomycetes* at dilution 10^{-2} (0.813 log cfu/ml) and the highest count at dilution 10^{-3} (0.602 log cfu/ml), other samples had varying colony counts, ranging from 0.879 to 0.881 log cfu/ml at

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dilution 10^{-2} and from 0.378 to 0.461 log cfu/ml at dilution 10^{-3}. A total of 30 *Actinomycetes* isolates were recovered based on their cultural characteristics (Table 2). The isolates displayed marked diversity in terms of pigment, colony shape, edge, and consistency, indicating variability in the environmental conditions at the collection sites.

Table 1: Mean number of colonies of *Actinomycetes* (Log cfu/ml) from different soil samples collected from Ikere-Ekiti

Isolates sources (n)	Dilution 10^{-2} (Mean cfu/ml)	Dilution 10^{-2} (Log cfu/ml)	Dilution 10^{-3} (Mean cfu/ml)	Dilution 10^{-3} (Log cfu/ml)
Stream sediments (18)	7.56	0.879	2.89	0.461
Loamy soil (18)	6.50	0.813	4.00	0.602
Dung soil (18)	7.61	0.881	2.39	0.378

Table 2: Cultural characteristics of selected *Actinomycetes* isolates from different soil samples

S/N	Sample	Edge	Pigment	Consistency	Elevation	Size	Shape	Surface
	SB2	Irregular	White with green	Leathery	Raised	Large	Round	Feathery
	SB3[1]	Round	Green with white	Dry	Low	Small	Round	Smooth
	SB3[2]	Round	Pink	Dry	Low	Small	Round	Smooth
	SC3[1]	Round	White	Leathery	High	Small	Round	Feathery
	SD3[1]	Round	White	Wavy	High	Small	Round	Feathery
	SE2[1]	Round	White	Leathery	High	Small	Round	Feathery
	SE2[2]	Round	Green	Dry	Low	Small	Round	Smooth
	DA3[2]	Wavy	White	Leathery	Low	Small	Round	Rough
	DB2[2]	Round	White	Leathery	Low	Small	Round	Rough
	DB3	Round	White	Leathery	High	Small	Round	Feathery
	DC2[2]	Wavy	White	Leathery	High	Small	Round	Feathery
	DC3	Wavy	White	Leathery	High	Small	Irregular	Feathery
	DC3[1]	Wavy	Green with white	Leathery	High	Small	I Irregular	Feathery
	DD2[1]	Wavy	White	Leathery	Low	Small	Irregular	Dry
	DD2[2]	Wavy	White	Leathery	High	Large	Round	Feathery
	DD2[3]	Wavy	White	Leathery	High	Large	Irregular	Feathery
	DE2[1]	Round	Green	Dry	Low	Small	Round	Dry
	DE3[1]	Round	Green	Chalky powdery	Low	Small	Round	Dry
	LD2[1]	Wavy	White and green	Dry	Low	Small	Irregular	Chalky
	LD3[2]	Irregular	White with Green	Dry	low	Small	Irregular	Dry
	LA5	Irregular	White and brown	Leathery	Flat	Large	Irregular	Leathery
	LA4	Irregular	Brown	Dry	Flat	Small	Irregular	Powdery
	LA2	Wavy	White	Dry	low	Small	Irregular	Chalky
	LA3	Irregular	Cream and Brown	Dry	Flat	Small	Irregular	Powdery
	LD2	Round	Cream with white	Dry	Low	Small	Round	Powdery
	DA1	Round	White	Dry	Low	Small	Round	Smooth
	LDI	Wavy	White	Leathery	High	Small	Round	Feathery
	DC1	Round	White	Chalky	Low	Small	Round	Dry
	DC2	Round	White	Chalky	Low	Small	Round	Dry
	LE3	Round	White/Green	Chalky	High	Small	Round	Powdery

Key: Stream A- SA, Stream B- SB, Stream C- SC, Stream D- SD, Stream E- SE, Loamy soil A - LA, Loamy soil - LB, Loamy soil C- LC, Loamy soil D –LD, Loamy soil E- LE, and Decomposed soils (Dung) A- DA, Dung B- DB, Dung C- DC, Dung D - DD, Dung E – DE. Superscript numbers indicate different isolates from the same source.

The Gram-staining tests show that all *Actinomycetes* isolates were Gram-positive, albeit with variations in cell shape and arrangement. Of the thirty (30) isolates of *Actinomycetes*, 85 % were catalase-positive, indicating their ability to tolerate oxidative stress, while 5 % of the isolates tested negative. Furthermore, 33 % of the isolates demonstrated positive indole production, while 67 % were negative, highlighting the biochemical variability within the isolates population. (Figure 2).

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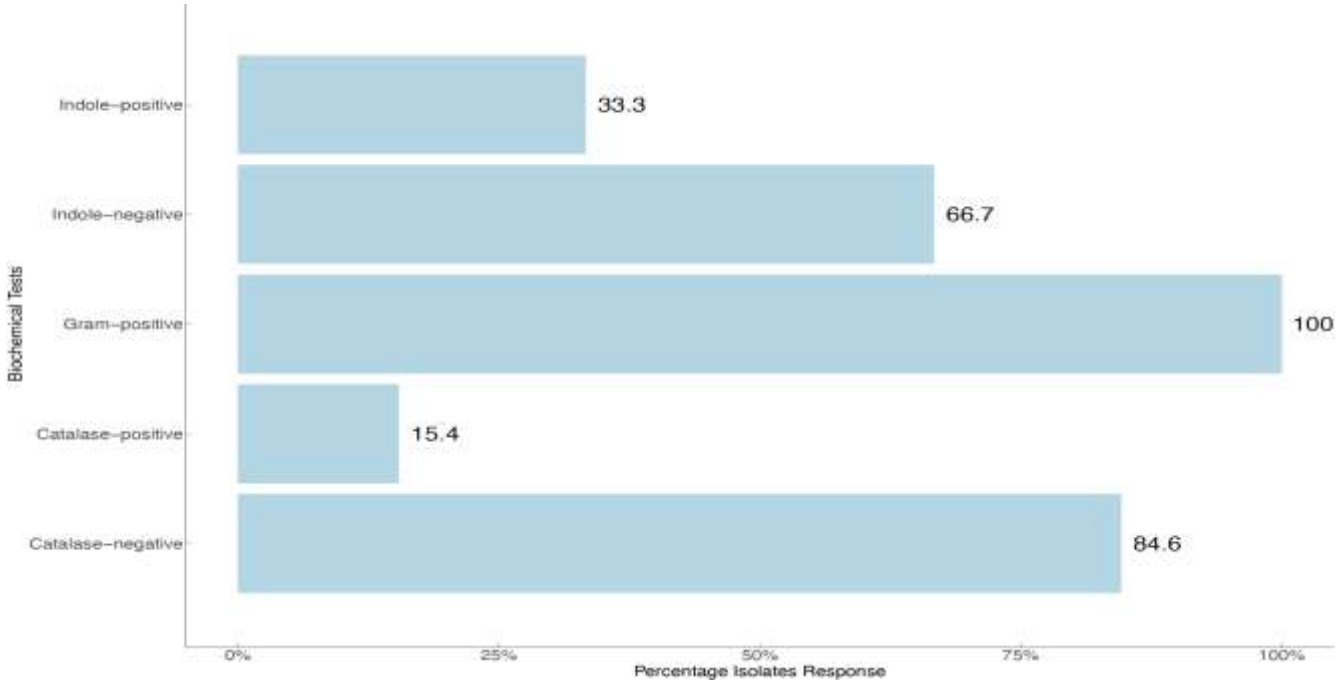

Figure 2: Gram staining and biochemical characteristics of the *Actinomycetes* isolates

Thirty (30) *Actinomycetes* isolates were tested for antibacterial activity against six pathogenic bacterial strains. Table 3 presents the results of the primary screening of *Actinomycetes* against the test bacteria. Among these, isolate SB3[1] demonstrated the highest antibacterial activity against all test organisms, while some isolates lacked antibacterial activity.

The secondary screening of antibacterial activity of the isolates is shown in Table 4. Strikingly, isolates SB3[1] and LD[1] had the highest activity against the four test organisms. These findings highlight the varying levels of potential for antibacterial activities among the isolates. In summary, 27 % of the *Actinomycetes* isolates had no inhibitory activity against any of the test organisms. In comparison, 50 % had activity against two isolates, and 23 % were only active against three or more bacterial pathogens.

For antifungal activity, 27 *Actinomycetes* were tested against two fungi. The primary antifungal screening revealed that 37 % of the *Actinomycetes* isolates exhibited inhibitory activities against the two test fungal pathogens (Table 5). In contrast, Table 6 shows that only *Aspergillus flavus* was susceptible to all the isolates during the secondary screening, except for isolate LE3.

The BOUESTI campus metropolis, our study area, is primarily an agricultural settlement where subsistence farming is the primary land use. It is therefore not uncommon to recover numerous *Actinomycetes* strains from different sample sources. The isolation of *Actinomycetes* from samples collected in this study confirms various studies reported on the isolation of *Actinomycetes* from other habitats. [12 - 14] Soil as a natural reservoir of microorganisms is saddled with the maintenance of key ecological processes via complex interaction between its microbiome and vital physicochemical parameters, as well as the local farming practices like ploughing and heaping during rainy/planting seasons, which may specifically increase the number of *Actinomycetes* via increased soil aeration, moisture and texture turnover, often shown by the distinctive, earthy scent of members of Genus *Streptomycetes* in newly tilled soil.[15 – 17] This scent is due to the production of volatile organic compounds (VOC) commonly known as geosmin, a secondary metabolite with a unique earthy scent that is detectable at low concentrations.[18, 19] Geosmin production and other VOCs produced by *Actinomycetes*, other than their metabolic activities significance, are also strongly linked to their other key, critical ecological roles.[20]

Studies on the examination and evaluation of *Actinomycetes* strains continue to provide a promising natural and environmentally sustainable source of antimicrobials effective against the challenge of antimicrobial resistance. [17, 21, 22] The existence and abundance of *Actinomycetes* in diverse soil and water habitats may also be contingent upon the physicochemical properties of these ecosystems. Essential physicochemical characteristics include pH, moisture, structure, temperature, salinity, oxygen levels, and chemistry.[9] These significant environmental elements, besides other anthropogenic factors, may affect the presence and abundance of *Actinomycetes* and any other group of microorganisms, resulting in fluctuations that impact the colony counts as observed in this study.

Our findings show that the isolated *Actinomycetes* exhibited distinct colony morphology on starch casein agar, as reported by Dwiyani et al. [13] and Patani et al. [23], which may be influenced by the soil type and the nutrients required to support their growth.[16] Biochemical tests are useful traditional physiology-dependent yardsticks for identifying microbial isolates based on their genetics and molecular profiles. [24] Here, biochemical tests are even more important in identifying *Actinomycetes* isolates because of their ability to produce bioactive secondary metabolites. Results from key biochemical tests, such as catalase and indole, are consistent with the study by Ajuzieogu et al. [25], who also reported varied responses to indole and catalase tests. The results demonstrate significant biochemical diversity and the intrinsic capacity of these *Actinomycetes* to withstand oxidative stress, which is crucial for adapting to their habitat and its characteristic fluctuating conditions.

The *Actinomycetes* exhibited diverse antibacterial activities, with isolate SB3[1] displaying the most potent broad-spectrum effects against all six test bacterial pathogens and significantly inhibiting *Klebsiella* sp., *Staphylococcus* sp., *Streptococcus* sp., and *Proteus* sp. (secondary screening inhibition zones of 11 mm, 8 mm, 8 mm, and 7 mm, respectively). However, other than the inhibitory activities of SB3[1] on *Escherichia coli* and *Pseudomonas* sp. in the primary screening, no zones of inhibition were observed in the secondary screening. Another *Actinomycetes* isolate, DB3, from secondary screening exhibited comparable broad-spectrum inhibitory effects against all isolates, with inhibition zones of 4 mm and 3 mm against *Staphylococcus* sp. and *Streptococcus* sp., respectively. Interestingly, the *Actinomycetes* isolate LD1 significantly inhibits *Staphylococcus* and *Streptococcus* species (inhibition zones of 8 mm and 4 mm, respectively), further projecting it as a potential candidate for treating infections caused by Gram-positive cocci. Findings from this study are consistent with those of a previous study on the antibacterial activities of *Actinomycetes* from the clove plant, which demonstrated significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.[26] Furthermore, our findings are similar to another study on the notable inhibitory activities of 18 extremophilic *Actinomycetes* on *S. aureus* [27]

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Table 3: Primary screening of the *Actinomycetes* for antibacterial activities against bacterial test organisms

S/N	Isolates	Test Organisms					
		Klebsiella sp.	*Staphylococcus* sp.	*Streptococcus* sp.	*E. coli*	*Proteus* sp.	*Pseudomonas* sp.
	LA2	-	++	++	-	-	-
	LA3	+	-	-	++	-	-
	LA4	-	++	-	-	-	-
	LA5	++	+	-	-	-	++
	LD1	-	++	++	-	-	-
	LD2	-	-	++	-	-	-
	LE3	+	-	-	-	-	++
	DA1	++	++	-	-	-	-
	DC1	-	-	-	-	++	-
	DC2	++	-	-	-	++	-
	SB2	-	-	-	-	-	-
	SB3[1]	++	+++	+	++	+	+
	SB3[2]	-	-	-	-	-	-
	SC3[1]	-	-	-	-	-	-
	SD3[1]	-	-	-	-	-	-
	SE2[1]	+	+	+	+	+	-
	SE2[2]	-	-	+	-	++	-
	DA3[2]	-	-	-	-	-	-
	DB2[2]	+	-	-	-	-	-
	DB3	+	+	++	+	+	+
	DC2[2]	-	-	-	-	-	-
	DC3	-	-	-	+	-	-
	DC3[1]	-	-	-	-	-	-
	DD2[1]	+	-	+	-	-	+
	DD2[2]	-	-	-	-	+	+
	DD2[3]	++	-	-	+	+	-
	DE2[1]	-	-	-	-	-	+
	DE3[1]	-	-	-	-	-	-
	LD2[1]	+	-	-	-	-	-
	LD3[2]	-	-	+	+	+	-

KEY: ++ means strong antibacterial activity; + means mild antibacterial activity; - means no antibacterial activity
Stream A- SA, Stream B- SB, Stream C- SC, Stream D- SD, Stream E- SE, Loamy soil A - LA, Loamy soil - LB, Loamy soil C- LC, Loamy soil D – LD, Loamy soil E- LE, and Decomposed soils (Dung) A- DA, Dung B- DB, Dung C- DC, Dung D - DD, Dung E – DE. Superscript numbers indicate different isolates from the same source.

Table 4: Zones of inhibition (mm) showing the antibacterial activities of the *Actinomycetes* isolates on bacterial test organisms during secondary screening

Test Organisms	Control	DB3	SB3[1]	SE2[1]	LE3	DC2	DA1	LA4	LD1	LA3	LA2
					Zones of inhibition (mm)						
***Staphylococcus* sp.**	0.0	4.0	8.0	12.0	20.0	7.0	0.0	6.0	8.0	18.0	11.0
***Streptococcus* sp.**	0.0	0.0	8.0	10	14.0	20.0	10.0	20.0	4.0	11.0	0.0
***Proteus* sp.**	0.0	3.0	7.0	4.0	0.0	0.0	0.0	0.0	4.0	0.0	7.0
E. coli	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
***Klebsiella* sp.**	0.0	0.0	11	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0
***Pseudomonas* sp**	0.0	0.0	0.0	0.0	19.0	15.0	0.0	18.0	8.0	0.0	0.0

Key: Stream A- SA, Stream B- SB, Stream C- SC, Stream D- SD, Stream E- SE, Loamy soil A - LA, Loamy soil - LB, Loamy soil C- LC, Loamy soil D –LD, Loamy soil E- LE, and Decomposed soils (Dung) A- DA, Dung B- DB, Dung C- DC, Dung D - DD, Dung E – DE. Superscript numbers indicate different isolates from the same source.

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Table 5: Antifungal activities of *Actinomycetes* against fungal test organisms (Primary Screening)

S/N	Isolates ID	*Aspergillus flavus*	*Aspergillus niger*
	DA1	+	++
	DA2	-	-
	DA3	++	++
	DB2	-	++
	DB3	-	-
	DC1	-	+
	DC2	+	++
	LA2	_	-
	LA3	-	++
	LA4	-	++
	LA4	++	-
	LA5	-	-
	LB1	-	-
	LBE3	-	-
	LC	-	-
	LD1	-	-
	LD2	-	-
	LE1	-	-
	LE2	++	++
	LE3	++	++
	SB3	++	++
	SB3[1]	++	++
	SC3[1]	++	++
	SC	++	++
	SC1[3]	-	++
	SD	-	-
	SE2	++	++

KEY: ++ means strong antifungal activity; + means mild antifungal activity; - means no antifungal activity; Stream A- SA, Stream B- SB, Stream C- SC, Stream D- SD, Stream E- SE, Loamy soil A - LA, Loamy soil - LB, Loamy soil C- LC, Loamy soil D –LD, Loamy soil E- LE, and Decomposed soils (Dung) A- DA, Dung B- DB, Dung C- DC, Dung D - DD, Dung E – DE. Superscript numbers indicate different isolates from the same source.

Table 6: Zone of inhibition (mm) showing Antifungal activities of *Actinomycetes* isolate on test fungi (Secondary screening)

S/N	Isolates	*Aspergillus flavus*	*Aspergillus niger*
	SB3	4	-
	DC2	9	-
	DA3	12	-
	SC3[1]	6	-
	SB3[1]	8	-
	LE2	9	-
	SE2[1]	11	-
	LE3	-	-
	SC	17	-
	Control	-	-

Key: Stream A- SA, Stream B- SB, Stream C- SC, Stream D- SD, Stream E- SE, Loamy soil A - LA, Loamy soil - LB, Loamy soil C- LC, Loamy soil D –LD, Loamy soil E- LE, and Decomposed soils (Dung) A- DA, Dung B- DB, Dung C- DC, Dung D - DD, Dung E – DE. Superscript numbers indicate different isolates from the same source.

The comparative antifungal activities of the *Actinomycetes* against the two test fungi species also revealed differing inhibitory activities, ranging from borderline to strong among the isolates. These differing inhibitory activities are attributable to the capability of the *Actinomycetes* strain to produce broad-spectrum antimicrobial compounds that are potent not only against Gram-positive and Gram-negative bacteria, but also fungi. Secondary screening revealed that approximately 90 % of the fungal isolates exhibited inhibition zones ranging from 4 to 17 mm against *Aspergillus flavus*. Of particular interest is isolate SC, which exhibits the most significant inhibition (a zone measuring 17 mm) against *A. flavus*, followed by isolates DA3 (12 mm) and SE21 (11 mm). These findings strengthen the documentation on the antifungal activities of *Actinomycetes* from various habitats, including the Arctic region, plant rhizospheres, and other extreme environments, against phytopathogenic fungi such as *Colletotrichu*m sp., *Fusarium oxysporum*, *Botrytis cinerea*, and *Candida albicans*.[21, 28 - 30] The production of antifungal agents specifically by *Actinomycetes* in the *Streptomycetes* genus is standard. Their various application potentials as natural biocontrol agents further provide significant economic opportunities and sustainable agricultural practices. Leveraging this research may be an excellent option for discovering novel bioactive compounds that can be useful for addressing the current menace of antimicrobial resistance.

Conclusion

The antimicrobial activity potential of the *Actinomycetes* isolates from this study, using both primary and secondary screening methods, has been established. Many of the *Actinomycetes* isolates elicited inhibitory activities against test organisms. However, a significant limitation is our inability to sequence and identify these strains. Further exploration of these *Actinomycetes*, including molecular identification and characterization of their secondary metabolites, could lead to the discovery of new antimicrobial agents. Given the increasing problem of antibiotic resistance, the continued search for natural antimicrobial compounds is crucial to sourcing *Actinomycetes* from local environments.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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