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Bioprospecting of Endophytic Actinobacteria from Selected Ethno-Medicinal Plants for Antibacterial and Anticancer Activities

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ABSTRACT

Endophytic actinobacteria are promising sources of secondary metabolites to combat antimicrobial resistance and cancer. **Objectives:** To isolate endophytic actinobacteria from ethnomedicinal plants and to evaluate their antibacterial, antibiofilm, and *in-vitro* anticancer potential. **Methods:** The endophytic actinobacteria were isolated from *Aloe barbadensis* and *Azadirachta indica* using the spread-plate technique on selective media. Purified isolates were screened for antibacterial activity by well diffusion assay and for general cytotoxicity by brine shrimp lethality assay. The crude methanolic extracts of bioactive strains were further tested against A549 lung carcinoma cells (MTT assay) and *Escherichia coli* biofilms and were chemically profiled by TLC and GC-MS. **Results:** Several endophytic actinobacteria isolates were recovered, many of which inhibited at least one test bacterium. The extracts of strains IEA2, IEA4, IEA5, IEA14, and IEA18 reduced A549 cell viability by approximately 32-87% at 0.1-1 mg/ml, indicating strong in-vitro anticancer potential. The extracts of isolates IEA2, IEA4, and IEA5 dispersed the *E. coli* biofilms by 85%, 87%, and 79% at 3µg/mL, demonstrating marked antibiofilm activity. GC-MS analysis of active extracts revealed multiple low-molecular-weight metabolites (≈100-500 Da), consistent with the production of diverse secondary metabolites by actinobacteria. **Conclusions:** Five strains reduced A549 viability by 32-87% and dispersed *E. coli* biofilms by 79-87%, indicating that aloe- and neem-derived endophytes are promising sources of compounds against drug-resistant bacteria and lungs cancer; however, these findings are based on crude extracts and need confirmation by purification and mechanistic studies.

INTRODUCTION

Plants are a rich source of bioactive compounds, which are produced by the bacteria that live inside the tissues. These endophytes proliferate and reside in plant tissues and excrete secondary metabolites, making them a valuable source for discovering new therapeutic agents [1]. Medicinal plants harbor unique actinobacteria in their tissues and rhizosphere. Due to the co-evolution with defense compounds, these actinobacteria often produce novel bioactive metabolites (antibiotics, anticancer agents, and antioxidants) that can be used for drug development. In the meantime, plants select useful microbes in the environment by means of root exudates in a symbiotic and selective interaction, stimulating ecological stability and evolutionary victories [2]. The endophytic association between plants and their microbes is important in guarding the host against pathogens and pests [3]. Moreover, endophytes are part of producing secondary metabolites that supplement the plant phytochemical profile and enhance its chemical diversity as a whole [4]. Actinobacteria are among these microbes, which exhibit a high level of affinity with the root tissues relative to other plant organs [5]. Multiple studies have reported structurally novel antibiotics and other metabolites from endophytic *Streptomyces* species in

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diverse hosts, underscoring their value for natural product discovery [6]. Community studies also reveal that populations of endophytic actinobacteria are mainly dominated by *Streptomyces* species in plant tissues, although other genera also make up a proportion of endophytic actinobacteria [7]. The endophytic actinobacterial genera *Glycomyces* and *Streptomyces* have been identified as promising sources of bioactive compounds effective against methicillin-resistant *Staphylococcus aureus* in a number of studies [8]. In addition to their antimicrobial action, endophytic actinobacteria may also produce plant growth-promoting compounds that induce growth and development in their hosts, establishing a symbiotic relationship between the microbe and the plant [9]. In one notable study [10], investigators found 398 Gram-positive endophytic actinomycete isolates with an antagonistic effect on phytopathogens, having a significant potential as a biocontrol agent in the sustainable management of plant diseases[11]. Furthermore, scientists have discovered new antibiotics in *Streptomyces* strains residing within the tissues of *Aucuba japonica*, including two novobiocin derivatives that had not been previously described [12]. Similarly, researchers have identified two novel butyrolactone antibiotics, cedarmycin A and B, produced by endophytic *Streptomyces* strains residing within *Cryptomeria japonica* plants [13]. Furthermore, a new naphthoquinone antibiotic, alnumycin, was identified in endophytic *Streptomyces* from *Alnus glutinosa*[14].

Despite increasing reports on endophytic actinobacteria as potential sources of novel bioactive compounds, limited studies have systematically explored ethnomedicinal plants from Pakistan for antibacterial, antibiofilm, and anticancer properties. In particular, Aloe barbadensis and Azadirachta indica remain under-investigated regarding the diversity and therapeutic potential of their endophytic actinobacterial communities. Furthermore, integrated evaluation combining antimicrobial, antibiofilm, cytotoxic, and metabolomic profiling approaches is scarce. Therefore, there exists a significant research gap in identifying and characterizing bioactive endophytes from these medicinal plants that may contribute to novel drug discovery. This study aimed to hypothesize that endophytic actinobacteria from Aloe barbadensis and Azadirachta indica may produce secondary metabolites with significant antibacterial, antibiofilm, and in-vitro anticancer activities, and that metabolomic profiling may yield some chemically diverse bioactive compounds.

METHODS

This was an experimental study conducted at the Department of Microbiology, University of Central Punjab, Lahore, and at the Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, from August 2023 to March 2024. Sixteen healthy samples of *Azadirachta indica* (neem) and *Aloe barbadensis* (aloe vera) were collected from nurseries in Lahore. Plant tissues (roots, stems, and leaves) were washed, surface sterilized with 75% ethanol (5 min), 1% NaOCl (20 min) and 10% $NaHCO_3$ (10 min), and rinsed with sterile water to remove surface microbes [15]. Sterilized tissues were macerated in 9.9mL sterile water, and 0.1mL homogenate was spread on starch casein nitrate agar (SCA). Plates were incubated at 28°C for 14 days. Colonies with typical actinobacterial morphology (dry, hard, earthy odor) were selected [16]. Isolates were characterized by colony morphology, pigment formation, and biochemical tests as per Bergey's Manual and genomic DNA was extracted using phenol-chloroform, and the 16S rRNA gene was amplified with primers 27F/1492R [17]. PCR conditions: 95°C denaturation, 53°C annealing, 72°C extension. Amplicons were purified, sequenced, and compared using BLAST on EzBioCloud. Sequences were deposited in GenBank, and a phylogenetic tree was generated in MEGA11 using neighbor-joining with 1000 bootstraps [18]. Metabolites were extracted by culturing isolates in broth for 7 days at 28°C, 150 rpm. Cultures were sonicated (10 min)[19], mixed with XAD-16 resin (4% w/v) overnight, centrifuged, and the resin pellets were extracted with methanol. Crude extracts were used in bioassays. Antibacterial activity against MRSA and *Bacillus subtilis* was tested by agar well diffusion [20]. Wells (6 mm) were filled with 50 µL extract; zones of inhibition were measured after incubation at 37°C for 16–24 h. TLC was performed on silica gel plates developed in 10% methanol/dichloromethane and visualized under UV (254/366 nm). Plates were stained with anisaldehyde-H_2SO_4 to detect metabolite classes. GC-MS analysis was performed using a Perkin Elmer Clarus 600 system with an Elite-5MS column. Oven: 40–300°C at 5°C/min. Compounds were tentatively identified via NIST20 (similarity ≥80%). Cytotoxicity screening was performed by exposing *Artemia salina* nauplii to extracts in DMSO; Mortality (M%) was calculated as: M (A-B-N)/(G-N) X 100. The mortality rate formula parameters are as follows: where A = number of dead larvae in the test well after 24 h, B = mean number of dead larvae in control Wells after 24 h, G = total initial larval population per well, and N = number of larvae dead before test initiation. Estimates were done in triplicate; data analyzed by one-way ANOVA in GraphPad Prism. Anticancer activity was evaluated on A549 cells using the MTT assay [21]. Cells were treated with extracts (0.5–3 mg/mL) for 24 h. Absorbance was measured at 570 nm; cytotoxicity was calculated relative to controls. Antibiofilm activity against *Escherichia coli* was assessed by crystal violet assay [22]. Biofilm reduction was calculated as: % Biofilm reduction = [1-OD Sample – OD negative Control/OD positive control -

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OD negative Control] X 100. Based on bioactivity and TLC/GC-MS profile, *Streptomyces sp*. IEA4 was scaled-up in 2 L of GYM broth. Fermented broth was extracted with methanol/XAD-16N, concentrated, and fractionated on silica gel into 18 fractions, combined into F1-and F3. Preparative TLC yielded 19 subfractions screened against *B. subtilis* and MRSA. No human or animal subjects were involved. A549 work followed BSL-2 guidelines.

RESULTS

A total of twenty-six endophytic actinobacterial strains were recovered from medicinal plant tissues on starch casein agar. Most colonies developed on starch casein KNO_3 agar (SCA) as compact, dry, embedded growth. Twenty-six morphologically distinct isolates were selected as putative actinobacteria for further characterization, biological screening and chemical profiling (Figure 1).

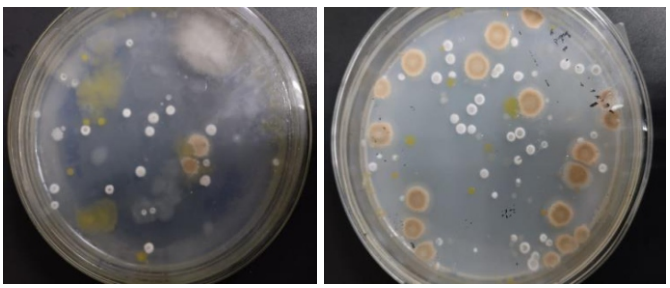

Figure 1: Spread Plate Method, Several Different Dry and Hard Actinobacterial Colonies with A Characteristic Earthy Aroma

Pure cultures of selected actinobacteria strains on the 7th day of incubation on SCA and GYM agar (Figure 2).

Figure 2: Pure Cultures of Selected Actinobacteria Strains on the 7th Day of Incubation on SCA And GYM Agar

The isolates were incubated on GYM and SCA agar for 7-10 days at 28 °C. Colony morphology on both media showed typical actinobacterial features, with rounded to irregular colonies, convex elevation, and hard, dry, or powdery textures. Gram staining confirmed all isolates as Gram-positive, filamentous bacteria with branching hyphae and chain-like spore arrangements. Strains from *Azadirachta indica* (e.g., IEA8, IEA14, IEA4) showed off-white to light-pink growth on SCA, whereas isolates from *Aloe barbadensis* (e.g., IEA5, IEA18) produced off-white, regular colonies. Each isolate displayed a characteristic combination of colony color and texture under the tested conditions (Table 1).

Table 1: Colony morphology of isolated endophytic actinobacterial strains on 7th day of incubation at 28°C on SCA agar

Sr. No.	Actinobacterial Strains	Size (mm)	Shape	Color	Opacity	Elevation	Margin	Texture	Surface	Soluble Pigment
1	IEA 1	2.0	Circular	Crimson	Opaque	Umbonate	Entire	Dry	Wrinkled	Light brown
2	IEA 2	1.0	Irregular	White	Opaque	Convex	Entire	Dry	Smooth	Light brown
3	IEA 3	1.5	Circular	Off white	Opaque	Raised	Entire	Dry	Rough	None
4	IEA 4	2.0	Irregular	Pink	Opaque	Raised	Entire	Dry	Rough	None
5	IEA 5	2.0	Irregular	Pink	Opaque	Raised	Entire	Dry	Smooth	None
6	IEA 6	1.5	Circular	Crimson	Opaque	Raised	Entire	Dry	Wrinkled	Light brown
7	IEA 7	0.5	Circular	Stone grey	Opaque	Raised	Entire	Dry	Rough	None
8	IEA 8	2.0	Circular	Dark black	Opaque	Raised	Entire	Dry	Wrinkled	Light brown
9	IEA 9	1.3	Circular	Crimson	Opaque	Raised	Entire	Dry	Smooth	Light brown
10	IEA 10	0.3	Irregular	Crimson	Opaque	Raised	Entire	Dry	Rough	Light brown
11	IEA 11	0.6	Irregular	Stone grey	Opaque	Raised	Entire	Dry	Rough	None
12	IEA 12	1.1	Irregular	Stone grey	Opaque	Umbonate	Entire	Dry	Smooth	Light brown
13	IEA 13	1.2	Irregular	Stone grey	Opaque	Convex	Entire	Dry	Wrinkled	None
14	IEA 14	2.0	Circular	Stone grey	Opaque	Umbonate	Entire	Dry	Smooth	None
15	IEA 15	0.3	Irregular	Yellowish brown	Opaque	Convex	Entire	Dry	Wrinkled	None
16	IEA 16	0.4	Irregular	Dark black	Opaque	Flat	Entire	Dry	Smooth	None
17	IEA 17	1.0	Circular	Yellowish brown	Opaque	Umbonate	Entire	Dry	Rough	Light brown
18	IEA 18	0.5	Irregular	Dark black	Opaque	Convex	Entire	Dry	Rough	Light brown
19	IEA 19	0.9	Irregular	Crimson	Opaque	Umbonate	Entire	Dry	Rough	None
20	IEA 20	2.0	Circular	Crimson	Opaque	Convex	Entire	Dry	Rough	None
21	IEA 21	0.7	Circular	Yellowish brown	Opaque	Flat	Entire	Dry	Rough	Light brown
22	IEA 22	1.0	Irregular	Stone grey	Opaque	Convex	Entire	Dry	Smooth	Light brown
23	IEA 23	0.6	Circular	Stone grey	Opaque	Raised	Entire	Dry	Wrinkled	Light brown

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24	IEA 24	0.3	Irregular	Yellowish brown	Opaque	Raised	Entire	Dry	Rough	None
25	IEA 25	0.6	Irregular	Dark black	Opaque	Raised	Entire	Dry	Smooth	None
26	IEA 26	1.0	Circular	Stone grey	Opaque	Raised	Entire	Dry	None	None

Biochemical and physiological tests (melanin, esculin, urease, citrate, and methyl red) further supported their assignment to actinobacteria. Overall, 61% of the strains were positive for the methyl red test, 57% were able to utilize citrate, and 69% showed urease activity (urea hydrolysis). Half of the isolates produced melanin on tyrosine agar, and 60% hydrolyzed esculin. Molecular identification based on 16S rRNA gene sequencing and neighbor-joining phylogeny (MEGA 11, 1000 bootstrap replicates) assigned most isolates to the genus *Streptomyces*. Representative strains IEA4, IEA8, and IEA14 clustered with the described *Streptomyces* species and were deposited in GenBank under accession numbers PQ269133, PQ267973, and PQ269150, respectively, confirming their actinobacterial identity and revealing close similarity to previously reported strains (Table 2).

Table 2: GenBank Accession Numbers of the Selected Endophytic Actinobacterial Strains and Their Similarities (Percentage) with Previously Reported Strains

Actinobacterial Strains	Sequences Submitted	Gen Bank Accession Numbers	Closely Related Taxa	Percentage Similarity
IEA4	913	PQ269133	*Streptomyces sp.*	100%
IEA8	938	PQ267973	*Streptomyces sp.*	100%
IEA14	938	Pq269150	*Streptomyces microflavus*	100%

Methanolic crude extracts from the 26 isolates were screened for antibacterial activity against MRSA and *Bacillus subtilis*. Seven strains (IEA2, IEA3, IEA4, IEA5, IEA8, IEA14, and IEA18) produced clear inhibition zones against at least one test organism. IEA4 and IEA5 showed the strongest activity, with IEA5 producing the largest zone (17 mm) against *B. subtilis*, while IEA8 and IEA14 produced zones of 10 mm and 15 mm, respectively. Crude extracts demonstrated significant *in-vitro* anticancer activity against the A549 lung carcinoma cell line. Notably, strain IEA14 reduced cell viability by ~87% at 1 mg/mL, indicating strong cytotoxic potential, while IEA4 induced moderate inhibition (40–50%). This dose-dependent cytotoxicity aligns with previous reports on *Streptomyces*-derived metabolites, such as doxorubicin and actinomycin D, which trigger apoptosis via DNA intercalation or topoisomerase inhibition. The variability among strains suggests differences in secondary metabolite profiles, possibly involving polyketides or non-ribosomal peptides known for anticancer activity. The antibiofilm activity of extracts IEA2, IEA4, and IEA5 against *E. coli* displayed marked concentration-dependence, with inhibition rising from 8–13% at 0.5 mg/mL to 79–87% at 3.0 mg/mL. The potent activity of IEA4 (87% inhibition at 3 mg/mL) may be attributed to metabolites interfering with quorum sensing or extracellular polymeric substance (EPS) synthesis, mechanisms previously reported for *Streptomyces* antibiofilm agents. Such dose-responsive biofilm disruption is consistent with studies on actinobacterial cyclic peptides and enzymes that degrade biofilm matrices. 3A: Actinobacterial activity determined by the well diffusion method; 3B: % inhibition of cell viability against A549 lung cancer cell line, and 3C: Dose-response curves for biofilm inhibition of *E. coli* by endophytic actinobacterial extracts (Figure 3).

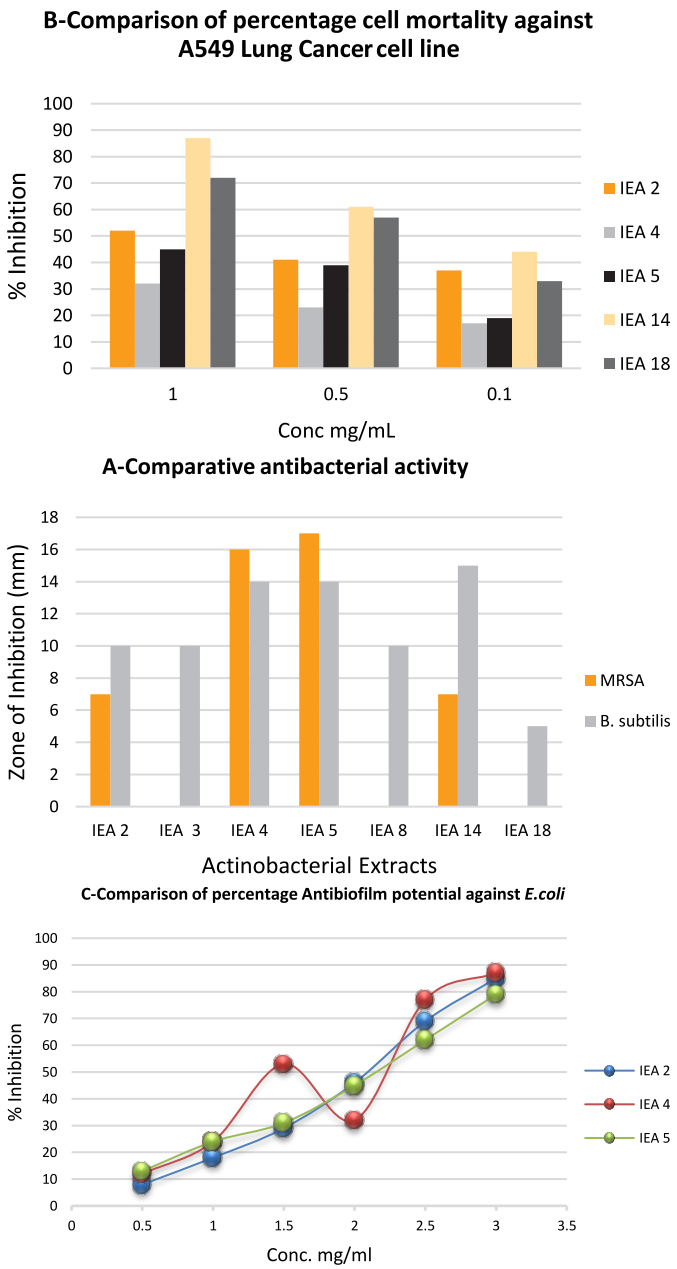

Figure 3: Evaluation of Bioactivity of Crude Extract

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Metabolomic profiles of the selected endophytic actinobacteria strains based on TLC and GC-MS analyses were done (Table 3).

Table 3: Metabolomic Profiles of the Selected Endophytic Actinobacteria Strains Based on TLC And GC-MS Analyses

Strains	Thin Layer Chromatography (TLC)			GCMS (tR/min)	Peak Area%	Molecular Formula	Molecular Weight	Compounds: Closest match in NIST library
	UV Visualization		Staining with Anisaldehyde / H2S04					
	254 nm	366 nm						
IEA 2	1	3	1 Greenish Band	24.891	18.64%	$C_7H_{10}N_2O_2$	154.17	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro
				35.053	5.91%	C_8H_{16}	97.09	1,2,4-trimethylcyclopentane
				–	–	$C_8H_{14}O_{16}$	366.19	cis-2,2,3,4-tetramethylcyclobutanone
				44.264	69.10%	$C_8H_{14}O$	126.20	Bis(2-ethylhexyl) phthalate
				–	–	$C_{24}H_{38}O_4$	390.55	Phthalic acid, di(2-propylpentyl) ester
				54.387	6.35%	$C_{10}H_{30}O_3Si_4$	310.68	Decamethyltetrasiloxane
				–	–	$C_{13}H_{19}NO_2$	222.30	4-(4-Hydroxy-2,5-dimethylbenzyl) morpholine
IEA 3	1	4	1 Yellowish Band	44.260	100%	$C_{24}H_{38}O_4$	390.55	Bis(2ethylhexyl) phthalate
IEA 4	1	1	1 Yellowish Band	44.255	34.09%	$C_{24}H_{38}O_4$	390.55	Bis(2-ethylhexyl) phthalate
				52.982	12.45%	$C_{20}H_{42}O_{11}$	458.54	Decaethylene glycol
				–	–	$C_{12}H_{24}O_6$	264.31	1,4,7,10,13,16-Hexaoxacyclooctadecane
				54.957	53.47%	$C_{20}H_{42}O_{11}$	458.54	Decaethylene glycol
				–	–	$C_{12}H_{24}O_6$	264.31	1,4,7,10,13,16-Hexaoxacyclooctadecane
IEA 5	1	4	1 Blackish Band	44.246	100%	$C_{24}H_{38}O_4$	390.55	Bis(2-ethylhexyl) phthalate
IEA 8	1	4	1 Greenish Band	–	–	–	–	–
IEA 14	1	4	1 Pinkish Band	44.248	100%	$C_{24}H_{38}O_4$	390.55	Bis(2-ethylhexyl) phthalate
						$C_{12}H_{14}O_4$	366.36	Di(Z)-hex-3-enyl phthalate
IEA 18	1	2	1 Brownish Band	–	–	–	–	–
IEA 20	1	1	1 Pinkish Band	–	–	–	–	–

Following primary screening, *Streptomyces* sp. IEA4 was scaled up to characterize its bioactive secondary metabolites. Column chromatography of the crude extract on silica gel with dichloromethane-methanol gradients generated 18 fractions, which were combined into three pooled fractions (F1-F3) based on TLC patterns. The three pooled fractions were tested against *B. subtilis*, *Klebsiella pneumoniae*, MRSA, and *E. coli*, and all showed varying degrees of activity. Further TLC analysis of F1-F3 revealed multiple differently colored bands (blue, green, purple, and light yellow; Supplementary Figure S6). Preparative silica gel TLC yielded 19 sub-fractions from F1-F3. These purified sub-fractions were evaluated against MRSA and *B. subtilis*; several retained measurable antimicrobial activities. The solvent control caused low mortality of *A. salina nauplii* (mean 25% ± SD). In contrast, most fractions increased mortality, with mean values ranging from ~33–63%. Several subfractions, particularly from F1-3 and the F2/F3 series, produced >60% mortality. Ordinary one-way ANOVA showed a significant overall effect of fraction on brine shrimp mortality ($F(19,40)=2.96$, $p=0.001$; $R^2=0.58$), with no evidence of unequal variances (Brown–Forsythe test, $p=0.699$). (A) Comparative anti-bacterial properties of pooled fractions F1-F3 against MRSA, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae* in the form of zone of inhibition (mm) using agar well diffusion methodology. (B) MRSA and *B. subtilis* inhibitory responses of eight preparative-TLC-purified sub-fractions of F1-F3. (C) Brine shrimp lethality of fractions and subfractions derived from *Streptomyces* sp. IEA4. Data were analyzed by ordinary one-way ANOVA followed by Dunnett's test versus control using GraphPad Prism 10; *p<0.050, **p<0.010 (Figure 4).

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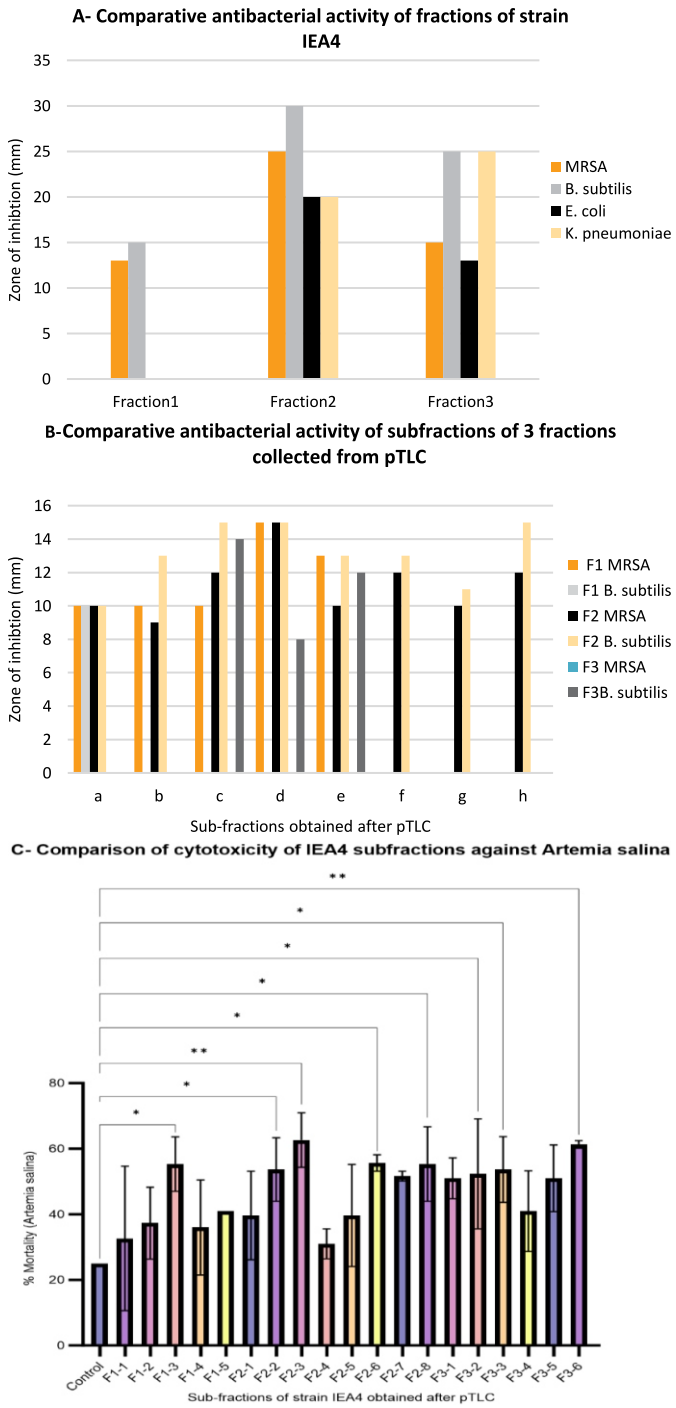

Figure 4: Fractions and Purified Sub-Fractions of *Streptomyces* Sp. IEA4 Bioactivity

DISCUSSION

Endophytic actinobacteria associated with medicinal plants in South Asia are increasingly recognized as valuable reservoirs of novel bioactive compounds due to their capacity to synthesize chemically diverse secondary metabolites with therapeutic relevance. In the present study, a total of 26 endophytic actinobacterial strains were successfully isolated from *Azadirachta indica* (neem) and *Aloe barbadensis* (aloe vera) following rigorous surface sterilization protocols. Preliminary identification based on colony morphology was carried out in accordance with the guidelines described in Bergey's Manual [23]. The isolated strains exhibited notable biological activities in multiple *in vitro* assays. Among them, strain IEA14 demonstrated the most pronounced anticancer activity, causing 87% inhibition of A549 lung carcinoma cells at a concentration of 1 mg/mL, which decreased to 44% at 0.1 mg/mL, indicating a clear concentration-dependent effect. Similarly, antibiofilm activity against *Escherichia coli* increased with extract concentration, with strain IEA4 showing the highest inhibition (87%) at 3 mg/mL, followed by IEA2 (85%) and IEA5 (79%). Furthermore, antimicrobial evaluation revealed that seven out of the 26 isolates exhibited strong inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* [24]. The observed variation in bacterial susceptibility is consistent with previous findings, which report higher sensitivity of Gram-positive bacteria to antimicrobial agents due to the lack of an outer membrane and structural differences in their cell walls. The dominance of *Streptomyces* species among the isolates aligns with global studies identifying this genus as the most prevalent endophytic actinobacteria in medicinal and agricultural plants [25]. The co-occurrence of *Micromonospora* and *Nocardia* further enhances the metabolic diversity of the isolated community, as these genera are known to produce distinct classes of antibiotics and anticancer agents [7]. This taxonomic profile mirrors recent findings by Saikia *et al.* (2022), who reported a similar distribution of actinobacterial genera in tropical medicinal plants and linked it to enhanced functional and chemical diversity within the plant microbiome [26]. Metabolomic analysis using thin-layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS) underscored the chemical richness of the bioactive isolates. TLC profiles displayed multiple UV-absorbing and fluorescent bands, and distinct staining with anisaldehyde/H_2SO_4 indicated the presence of terpenoids, alkaloids, and phenolic compounds—classes widely associated with bioactivity.GC-MS tentatively identified several low-molecular-weight metabolites (<500 Da), including phthalate derivatives and heterocyclic scaffolds, consistent with prior reports of similar compounds in endophytic actinobacterial extracts [27]. While these identifications are preliminary and require confirmation through advanced spectroscopic techniques such as NMR and HRMS, the observed chemical diversity reaffirms the biosynthetic potential of these endophytes. Further cytotoxicity assays on normal cell lines would help establish the therapeutic index of these promising

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metabolites.

This study is limited by the use of crude extracts and in-vitro bioassays, which do not fully elucidate the specific active compounds or their precise mechanisms of action. GC-MS identification was tentative and requires confirmation through advanced spectroscopic techniques such as NMR and HRMS. Additionally, cytotoxicity was evaluated on a single cancer cell line without comparison to normal cell lines, limiting assessment of selectivity and therapeutic index. Future studies should focus on bioassay-guided purification, structural elucidation, whole-genome sequencing of promising strains, and in-vivo validation to advance these endophytic actinobacteria as potential therapeutic candidates.

CONCLUSION

This study screened endophytic actinobacteria from the ethnomedicinal plants *Aloe barbadensis* and *Azadirachta indica* for growth inhibition of drug-resistant bacteria and *in-vitro* anticancer activity. Twenty-six strains, dominated by *Streptomyces* but also including *Micromonospora* and *Nocardia*, were obtained, and several produced crude extracts with strong activity against MRSA and *B. subtilis* as well as notable cytotoxic effects on A549 lung cancer cells. Metabolomic profiling indicated that these bioactive isolates synthesize a chemically diverse set of low-molecular-weight metabolites consistent with secondary metabolite production. Overall, endophytic actinobacteria from aloe and neem represent a promising source of antibacterial, antibiofilm and anticancer candidates and warrant further bioassay-guided purification, structure elucidation, and genome-guided discovery of novel therapeutic compounds.

Authors' Contribution

Conceptualization: IS[1], NN, IS[2]
Methodology: IS[1], AI, NN, IS[2]
Formal analysis: AI, IS[2]
Writing and Drafting: IS[1], IS[2]
Review and Editing: AI, IS[1], IS[2], NN

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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