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Research Article

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FERMENTATION, PURIFICATION AND *IN-VITRO* EVALUATION OF ANTIMICROBIAL, ANTITUMOR AND ANTIOXIDANT COMPOUNDS FROM A HIMALAYAN ACTINOBACTERIA STRAIN *STREPTOMYCES* SP. PU-AK14

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ABSTRACT

This study reports the laboratory scale fermentation, compounds purification and bioactivity screening of the strain *Streptomyces* sp. PU-AK14 isolated from Himalayan mountains range, Pakistan, for its potential to produce antimicrobial, antioxidant and antitumor compounds. For this purpose, laboratory fermentation (20 L working volume) was performed followed by extraction of the compounds by XAD adsorbent gel using the solvents methanol and ethyl acetate. Further purification of the compounds was done by silica gel column chromatography, sephadex column and preparative TLC. A total of 18 partially purified compounds were retrieved from crude extract and were screened for biological activity. The crude extract was more active against gram negative bacteria with MIC of 0.1 mg/ml against *Klebsiella pneumoniae* and *Escherichia coli.* Eight of the partially purified compounds exhibited activity against MRSA whereas five fractions were active against gram negative bacteria. Seven partially purified fractions exhibited promising antioxidant activity with radical scavenging activity ranging from 78.2 to 42.34 percent. Cytotoxicity of partially purified compounds ranged from 75% to 100% larval mortality at 100mg/ml against *Artemia salina* and 50% to 52% cell mortality against HCT 116 colorectal cancer cell line at 100mg/ml. The best anti-tumor activity was exhibited by the fraction AK141111L. The study suggested that *Streptomyces* sp. PU-AK14 is a potential source of chemotherapeutically useful compounds.

Keywords: *Himalayan actinobacteria, Streptomyces ansochromogenes PU-AK14, bioactive secondary metabolites, metabolic profiling.*

INTRODUCTION

Actinobacteria are medically significant group that produce a wide variety of biologically active secondary metabolites, *i.e.,* antimicrobial, antioxidant, anti-tumor, immunosuppressants, antiviral and insecticidal compounds (El-Naggar, 2021; Subbarao *et al*., 2010). These secondary metabolites are not essential for bacterial growth, but they are crucial for bacteria to compete with other living organisms for their survival or to facilitate the defence mechanism of host (Balachandar *et al*., 2018). Sampling from unexplored region is an important key to discover novel bacteria with distinctive biological activities (Wasito, 2020). The Himalayan range of Pakistan latitude: 35.24674°N and longitude: 74.55322°E is underexplored region for detection of bioactive secondary metabolites from actinobacteria. The vast Great Himalayas is approximately 1400 miles, with an average elevation of 6000 meters and housing the world's tallest peaks. These mountains form a natural barrier between central and south Asia, covering India, Nepal, Pakistan, and part of China (Cheema *et al*., 2021). The high elevations of the Himalayas create challenges like little vegetation, cold weather and low nutrition levels. For survival in such adverse environment, *Streptomyces* produce antimicrobials and regulate their chaperone system to maintain the proteins integrity (Bhat *et al*., 2024). There are several reports on the isolation and exploration of actinobacteria from Himalayas, researchers on both sides of the border in Pakistan and Indian territory of Himalayas have collected samples and have isolated and screened various *Streptomyces* species for the production of medicinally useful metabolites. Such as 4 new amino nucleosides, puromycin B-E were isolated and identified from a Himalayan *Streptomyces* sp. PU-14G by our research group in collaboration with a US research group (Abbas *et al*., 2018). In the same study new phenazine compounds; Baraphenazines A-G were discovered from another Himalayan *Streptomyces* sp. PU-10A (Wang *et al*., 2019). In another study the same research group reported the extensive screening of streptomycetes from Himalayan collection sites and identified a number of different metabolites by HPLC-MS technique based on comparison of the data with various databases, along with the laboratory scale fermentation, compounds purification and structure elucidation by mass spectrometry and NMR

spectroscopy from the selected *Streptomyces* sp. PU-MM93, where a number of well-known antibiotic and cytotoxic compounds such as the anthracycline, aranciamycin, aglycone SM-173B, Taurocholic acid, pactamycate, cyclo(L-Pro-L-Leu) and the neuroprotective carboxamide Oxachelin C (Cheema *et al*., 2021).

The *Streptomyces* species *Streptomyces ansochromogenes* investigated in this study has already been reported as a frequent producer of various bioactive metabolite such as Tylosin, Nikkomycin and the antitumor compound L-asparaginase etc (da Silva Lacerda *et al*., 2018; Kirienko, 2021; Li *et al*., 2021; Li *et al*., 2022). The whole genome sequencing and genome mining of the *Streptomyces ansochromogenes,* reported by Xu et al. 2017, showed the presence of 35 antibiotics biosynthetic gene clusters (BGCs) in the genome of the species.

This study aimed at the laboratory scale fermentation, screening and investigation of the Himalayan *Streptomyces* sp. PU-AK14, the strain was identified as *Streptomyces ansochromogenes* based on phenotypic, microscopic, biochemical characteristics and on the *16S rRNA* gene sequencing and sequence similarity with the type strain. The 20L laboratory fermentation and subsequent solvent extraction of the liquid broth and solid phase by the XAD adsorbent gel using methanol as the extraction solvent yielded a sufficient quantity of the crude extract which allowed the column fractionation and purification of bioactive metabolites by manual column chromatography, pTLC and HPLC-UV. A promising response of the production of antimicrobial, antitumor and antioxidant compounds has been observed in the Himalayan strain *Streptomyces* sp. PU-AK14.

MATERIALS AND METHODS

Taxonomic Characterization of Strain: The *Streptomyces* strain PU- AK14 was collected from culture collection of the Institute of MMG for isolation and partially identification of its secondary metabolites. Identification of the strain was based on morphological, biochemical, and genetic characterization as described by Gautham *et al.* (2012). The *16S rRNA* gene was amplified via PCR using the 27F forward primer (5'-AGAGTTTGATCCTGGCTCAG-3') and the 1522R reverse primer (5'-AAGGAGGTGATCCA(AG)CCGCA-3') (Naseer *et al.,* 2022).

Fermentation, Fractionation and Partial Purification of Components: The strain was cultivated on large scale (20 litres), with subsequent separation of cell mass and culture broth on the celite bed filter. The mycelium (solid phase) and culture media (liquid phase) were treated as described by Naseer *et al*. (2022). The combined crude extract (solid phase and liquid phase) was subjected to silica gel column fractionation (silica gel mesh 70-230 and particle size 0.063-0.2 mm). Elution of column was done by a gradient solvent mixture of dichloromethane and methanol (starting with 0% to 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and up to 100 % methanol) (Aftab *et al.,* 2015). The fractions were analysed using analytical TLC in combination with staining reagents, HPLC/UV and further purification was carried out by preparative TLC and Sephadex LH-20 (MeOH, 2.5× 45 cm) column (Sajid *et al*., 2011).

Antimicrobial Activity and Cytotoxicity Screening: The agar well diffusion method was employed to assess the antimicrobial activity of both crude extracts and fractions (Chelvan *et al*., 2016). Minimum inhibitory concentration (MIC) was measured by resazurin-based turbidimetric assay as explained by Teh *et al.*, (2017). The cytotoxicity of crude extracts and fractions was determined against *Artemia salina* by microwell cytotoxicity assay as described by Sajid *et al.,* (2009).

In-Vitro **Antitumor Activity:** The antitumor activity of extracts and fractions was evaluated by the MTT (3-(4,5-Dimethylthiazol-2-YI)-2,5-Diphenyltetrazolium Bromide) Assay and the Sulforhodamine B (SRB) assay as mentioned by Jubeen *et al.,* (2019) and Naseer *et al*., (2022).

In-Vitro **Antioxidant Activity:** The DPPH radical scavenging assay and the Nitric oxide scavenging assay was used for evaluation of antioxidant potential as described by Naseer *et al.,* (2022). Whereas phosphomolybdenum reduction test was used to measure total antioxidant activity as explained by Prieto *et al*., (1999).

RESULTS

Taxonomic Characterization: The *Streptomyces* sp. PU-AK14 colony displayed an irregular shape with undulating margins, deeply embedded in solid agar. It measured 4 mm with brown substrate mycelium, white aerial spores, and brown pigment formation. Microscopic examination showed that the strain was Gram-positive, filamentous bacteria, characterized by well-developed vegetative hyphae. *Streptomyces* sp. PU-AK14 exhibited positive results for melanin formation, trisodium citrate utilization, sodium lactate utilization, and hydrolysis of esculin, while it exhibited negative results for sodium malate utilization, oxalate utilization, and organic acid formation. It exhibited growth on all tested sugars and utilized most as its sole carbon source, except for L-arabinose and meso-inositol. In the BLAST analysis of the 16S rRNA gene sequence, *Streptomyces* sp. PU-AK14 exhibited a 99% similarity to *Streptomyces ansochromogenes*. The gene sequence data has been submitted to NCBI GenBank under accession number MH393205. The neighbor-joining phylogenetic tree, constructed using 16S rRNA gene sequences by MEGA X software with 1000 bootstrap replicates, illustrated a close relationship of the strain with the *Streptomyces ansochromogenes* genus (see Figure. 1 and Table 1) (Sijilmassi *et al*., 2020).

Fractionation and Partial Purification of Bioactive Compounds: The extracts obtained from both the solid

(9.05g) and liquid (49.67g) phases of *Streptomyces* strain AK14 displayed identical chemical profiles on the TLC plate. Therefore, SPE and LPE were combined, as they exhibited no discernible differences in their chemical composition. A total of 18 fractions were recovered from *Streptomyces* strain AK14 by silica gel column fractionation and were further used for biological screening and chemical profiling (Figure.2).

Analysis of Partially Purified Compounds for Chemical Profiling:

Thin Layer Chromatography analysis: The TLC analysis of the solid phase extract from Streptomyces sp. PU-AK14 revealed the presence of mostly less polar compounds, identifiable by their orange and purple color when observed under visible light. Additionally, under short UV (254nm), the TLC displayed prominent black coloured low polar compounds. Bands of varying polarities polar, medium polar, and less polar were visible under long UV (366nm) and with staining using anisaldehyde/H_2SO_4. On the contrary, the liquid phase extract exhibited a limited profile, with only five visible bands. Among these, three bands were observable under long UV (366nm) and two bands were evident under short UV (254nm).

High Performance Liquid Chromatography Analysis (HPLC-UV analysis): The evaluation of HPLC-UV chromatogram of crude extract exhibited diverse compounds with significant peaks at t_R 2.53, 2.88, 3.76, 4.10, 4.46, 4.84 and 5.94 min retention time with area of 20530, 34921, 9373, 6851, 5732, 8329 and 23001 mV.s respectively (Fig. 3 B1). The partially purified compound AK1410(14)1L showed major peaks at 3.24, 4.12 and 5.70 min retention time with peak area of 3190, 25103 and 8987 mV.s respectively. The fraction AK1410(14)1S showed major peak at 2.34, 3.48 and 4.06 min retention time with peak area of 1903, 21688 and 3066 mV.s respectively. The fraction AK1410(57)1L showed major peaks at 2.29, 2.65, 3.23 and 15.44 min retention time with peak area of 1053, 3196, 2738 and 7478 mV.s respectively. The fraction AK1410(57)2L showed major peaks at 2.24, 3.09 and 4.20 min retention time with peak area of 1549, 27413 and 2337 mV.s respectively. The fraction AK1410(57)3S showed major peaks at 2.66, 3.34 4.36, 4.84 and 5.38 min retention time with peak area of 4465, 57154,10508,50207 and 2285 mV.s respectively. The fraction AK1420(15)1L showed major peaks at 2.23, 2.52, 3.22, 4.37 and 5min retention time with peak area of 360, 282, 1452, 214 and 839 mV.s respectively. The fraction AK1420 (15) 2S showed major peak at 2.93min retention time with peak area of 7865 mV.s (Figure. 3 B2). The fraction AK141111L showed major peaks at 3.17 and 4.26 min retention time with peak area of 15914 and 1568 mV.s respectively. The fraction AK143050F1S showed major peaks at 2.53 and 3.07 min retention time with peak area of 13044 and 13718 mV.s respectively. The fraction AK143050F2S showed major peaks at 2.53 and 2.86 min retention time with peak area of 12338 and 22055 mV.s respectively. The fraction AK145060F1S showed major peaks at 2.24 and 2.83 min retention time with peak area of 23003 and 14580 mV.s respectively. The fraction AK145060F2S showed major peak at 2.7 min retention time with peak area of 109142 mV.s. The fraction AK1420302S showed major peaks at 2.92 and 4.57 retention time with peak area of 3910 and 805 mV.s respectively. The fraction AK1420 (15)1S2 showed major peak at 3.01 min retention time with peak area of 20502 mV.s.

Table 1: Characteristics of *Streptomyces* sp. PU-AK14.

Characteristics	***Streptomyces* sp. PU-AK14**
Colony shape	Irregular
Colony size (mm)	4
Colony margin	Undulate
Colony texture	Hard
Substrate color	Brown
Aerial color	White
Pigmentation	Brown
Production of melanin	+++
Utilization of Tri-sodium citrate	+
Utilization of Sodium malate	-
Utilization of Sodium lactate	+++
Utilization of oxalate	-
Formation of organic acids	-
Esculin hydrolysis	+++
Carbohydrate utilization	
D-glucose	+++
Sucrose	++
D-fructose	++
D-mannitol	+
L-arabinose	-
D- xylose	+
meso-inositol	-
16S rRNA sequencing	

Nucleotide length	558
%age homology	99%
Organism	*Streptomyces ansochromogenes*
GenBank Accession Number	MH393205

(+++) indicated excellent results, (++) indicated good results, (+) indicated moderate results and (–) indicated negative results.

Figure 1: Phylogenetic tree depicting the evolutionary relationship between *Streptomyces* sp. PU-AK14 and the reference sequences by BLAST. The tree was generated by Neighbor-Joining method, using *16S rRNA* gene sequence. The tree was constructed by MEGA X software using 1000 bootstrap replicates.

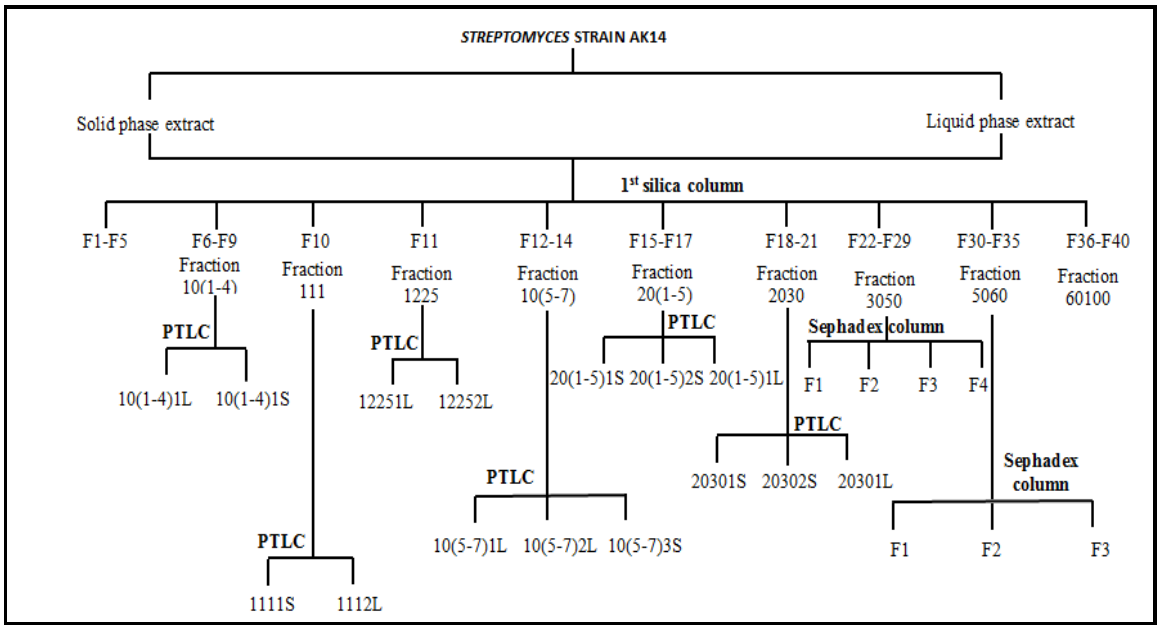

Figure 2: Work-up scheme for compounds purification from *Streptomyces* sp. PU-AK14.

Antimicrobial Efficacy and Cytotoxic potential: The *Streptomyces* sp. AK14 demonstrated a highest activity against gram negative organism such as *Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa* resulting in zone of inhibitions of 16mm, 13mm and 13mm respectively by agar well method. On the contrary, its effectiveness against gram-positive bacteria MRSA, *Bacillus subtilis*, and *Staphylococcus aureus* exhibited 10mm zone of inhibition (Fig. 4A). Various fractions AK14101, AK14104, AK14105, AK14106, AK14107, AK14201, AK14403, AK14406, AK14504, AK14505, AK14506 and AK14601 exhibited potent antimicrobial activity against MRSA, *Klebsiella pneumoniae*, and *Escherichia coli*,

producing zone of inhibitions ranging from 13 to 17mm (Fig. 7). The fractions such as AK1410(1-4)1L, AK1410(1-4)1S, AK141112L, AK1412252L, AK1410(5-7)1L, AK1410(5-7)3S, AK1420(1-5)1S, AK1420(1-5)2S, AK1420301L, and AK143050F1S displayed potent antimicrobial activity against MRSA and *Klebsiella pneumoniae*, with percentage inhibition ranged from 90 to 72 percent, as measured by microtiter plate assays.

The *Streptomyces* sp. PU-AK14 exhibited the lowest Minimum Inhibitory Concentration (MIC) against *Klebsiella pneumoniae* and *Escherichia coli* at 0.1mg/ml. Whereas, the MIC against *Bacillus subtilis*, *Staphylococcus haemolyticus*, *Staphylococcus aureus,* *Pseudomonas aeruginosa,* and *Proteus vulgaris* ranged from 0.8 to 3.2 mg/ml, as outlined in Table 2.

The crude extract, SPE, and LPE derived from *Streptomyces* sp. PU-AK14 demonstrated eminent cytotoxicity, reaching up to 84.67%, 81.81%, and 66.67% respectively at 100mg/ml against *Artemia salina* (Fig. 4B). The partially purified fractions, AK143050F1S exhibited 100% cytotoxicity at 100mg/ml concentration, whereas fraction AK143050F2S displayed a high cytotoxic effect of 99.8% at 100mg/ml. The other fractions, including AK145060F2S and AK1410(1-4)1S, also demonstrated potent cytotoxic activity (Figure. 6A).

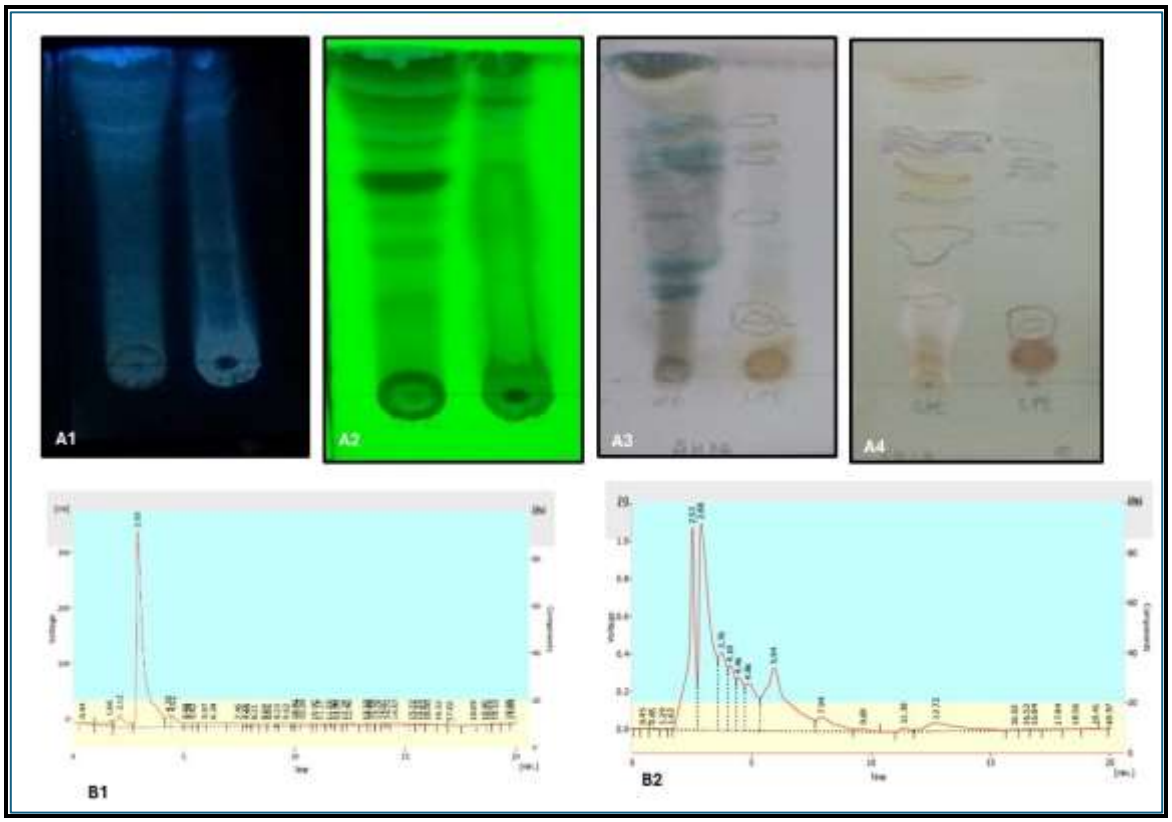

Figure 3. A) Thin Layer Chromatogram of solid and liquid phase extracts of *Streptomyces* sp. PU-AK14; A1: visible at 366 nm; A2: visible at 254 nm; A3: by staining with Anisaldehyde reagent; A4: under visible light; B1: HPLC-UV chromatogram of crude extract; B2: HPLC-UV chromatogram of Fraction AK1420(15)2S.

Table 2: MIC values of crude extracts of *Streptomyces* sp. PU-AK14 against pathogenic bacterial strains.

Bacterial strains	**MIC value (mg/ml)**
	PU-AK14
P. vulgaris	1.6
P. aeruginosa	3.2
E. coli	0.1
K. pneumoniae	0.1
S. haemolyticus	0.8
B.subtilis	0.8
S.aureus	1.6

In-vitro **Antitumor potential:** The extract from *Streptomyces* sp. PU-AK14 demonstrated significant cytotoxic effects against the HCT116 colorectal cancer cell line, with 66.78% cell mortality via the MTT assay and 73.10% cytotoxicity via the SRB assay. Both the SPE and LPE of *Streptomyces* sp. PU-AK14

demonstrated prominent cell mortality, with SPE at 73.29% and the LPE at 71.89% cell mortality at 100mg/ml (Fig. 4C). The partially purified fraction AK141111L exhibited the highest cellular mortality, reaching 80.51%, with an IC50 value of 74.29 (Figure. 6B).

In-vitro **Antioxidant potential:** The extract of *Streptomyces* sp. PU-AK14 demonstrated 53.39% inhibition by DPPH and 48.98% inhibition by nitric oxide. Meanwhile, the solid phase extract displayed 52.99% inhibition by DPPH and 46.99% inhibition by nitric oxide, and the liquid phase extract demonstrated 58.89% inhibition by DPPH and 59.18% inhibition by nitric oxide at 6.4 mg/ml (Fig. 4D and 4E). Seven retrieved fractions AK143050F1S, AK143050F2S, AK145060F1S, AK145060F2S, AK1410 (1-4)1L, AK1410 (1-4)1S and AK1420(1-5) 2S exhibited prominent antioxidant activity, with Inhibitory percentage ranged from 78.20 to 42.34% (Fig. 6C). The evaluation of antioxidant potential in terms of ascorbic acid equivalents (mg/ml) at 6.4mg/ml, showed values of 1.99, 1.738, and 2.945 ascorbic acid equivalents (mg/ml) of the crude extract, SPE and LPE respectively (Table 3).

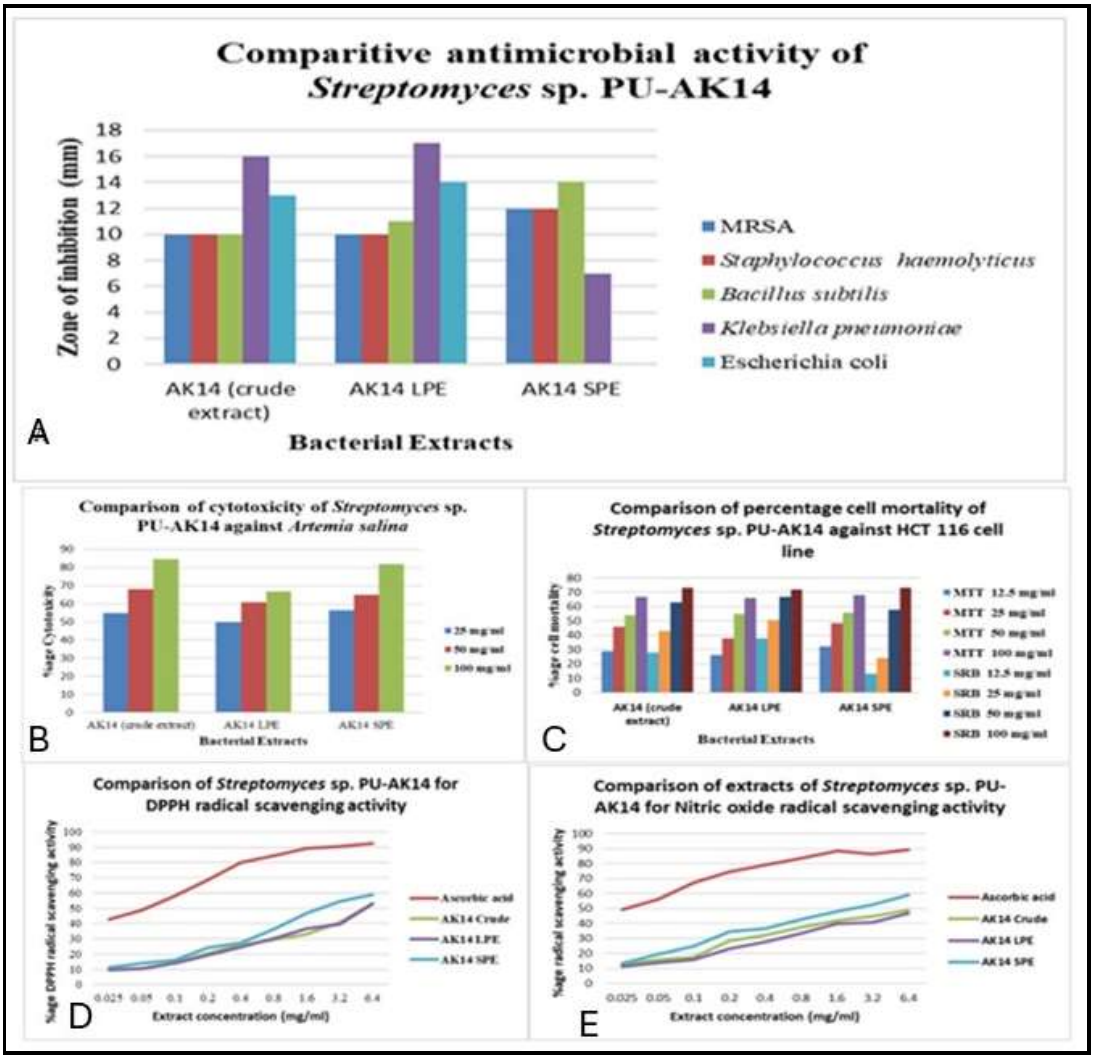

Figure 4. Evaluation of bioactivity of crude extract, LPE and SPE of *Streptomyces* sp. PU-AK14; A: Antibacterial activity determined by agar well method; B: Percentage larval mortality of *Artemia salina* by microwell cytotoxicity assay; C: Percentage growth inhibition by MTT and SRB assays on HCT 116 colorectal cancer cell line; D: Antioxidant potential by DPPH antioxidant assay; E: Antioxidant potential by NO scavenging assay.

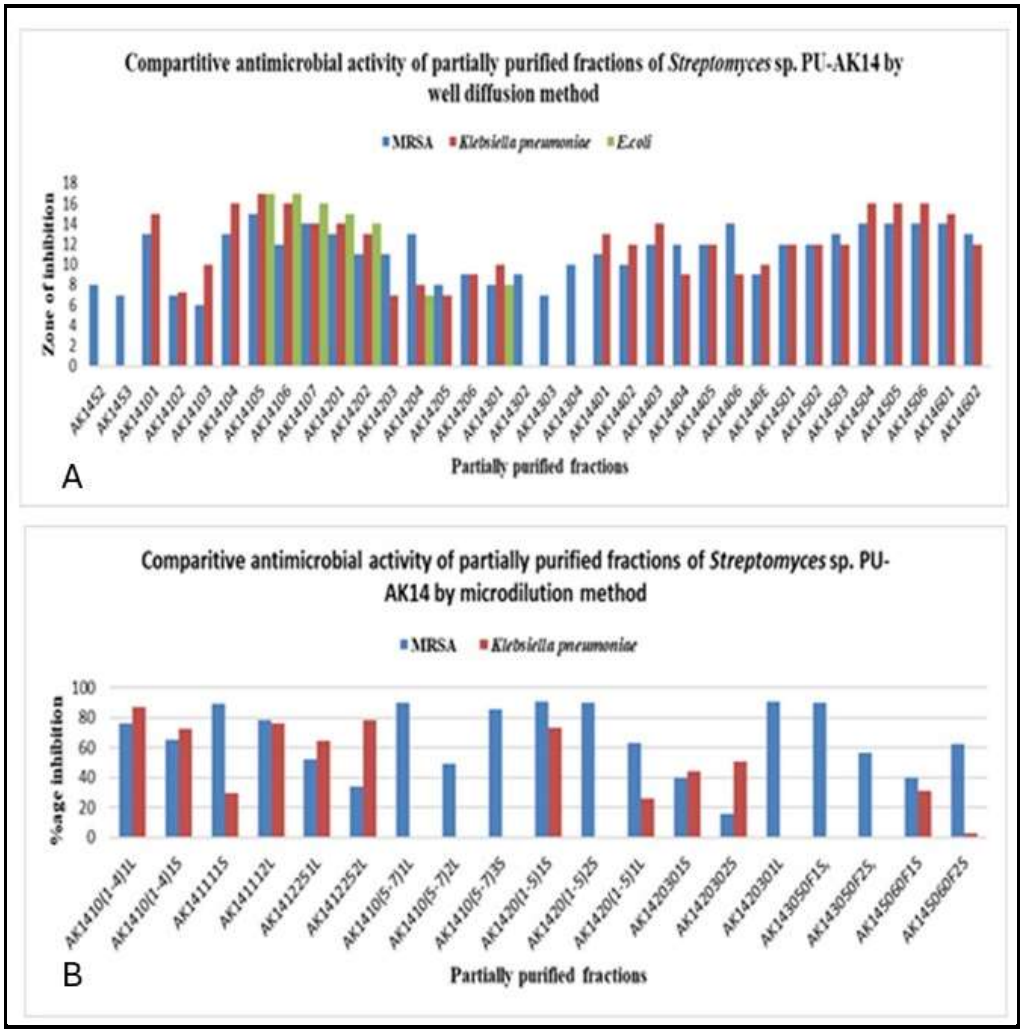

Figure 5. Evaluation of bioactivity of retrieved fractions from *Streptomyces* sp. PU-AK14; A: Antimicrobial assessment by agar well method; B: Antimicrobial activity by microtiter plate assay.

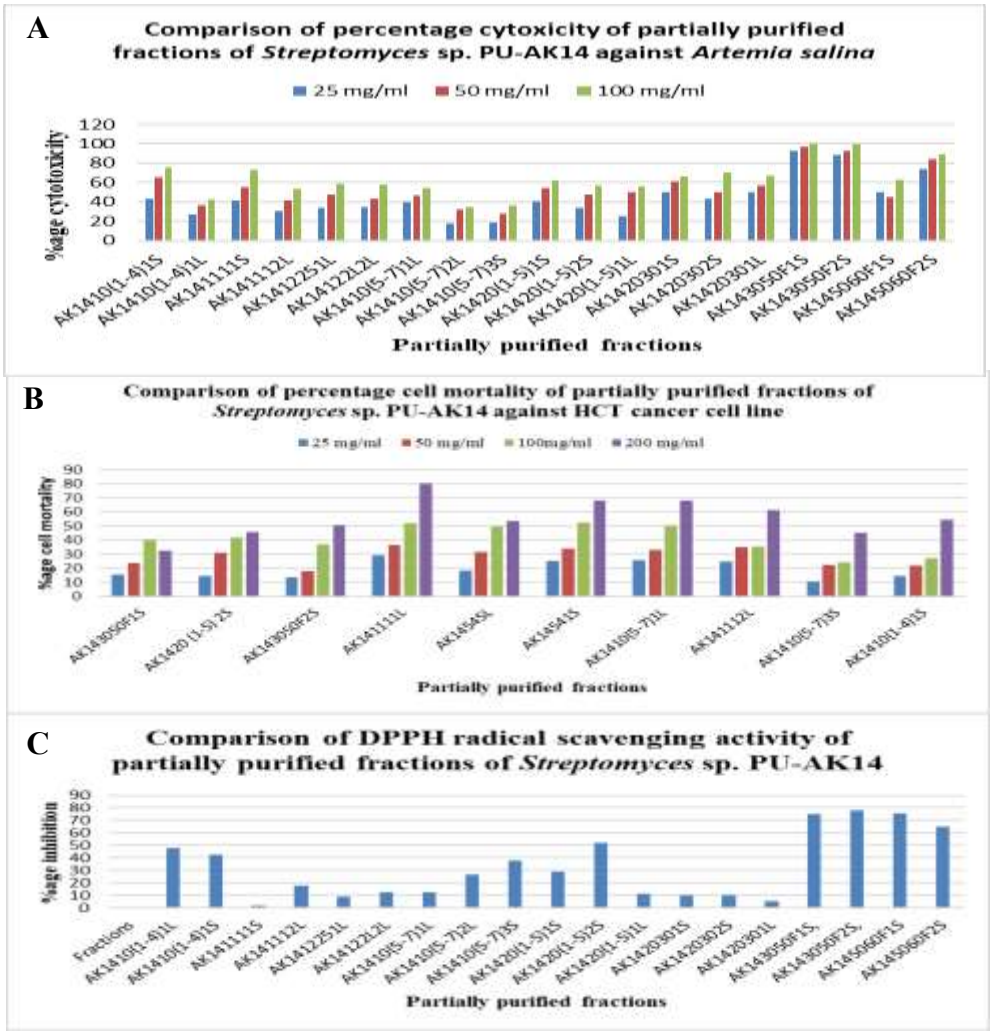

Figure 6. Evaluation of bioactivity of retrieved fractions from *Streptomyces* sp. PU-AK14; A: Cytotoxicity against *Artemia salina;* B: Percentage growth inhibition by MTT assay against HCT 116 colorectal cancer cell line; C: Antioxidant activity by DPPH antioxidant assay

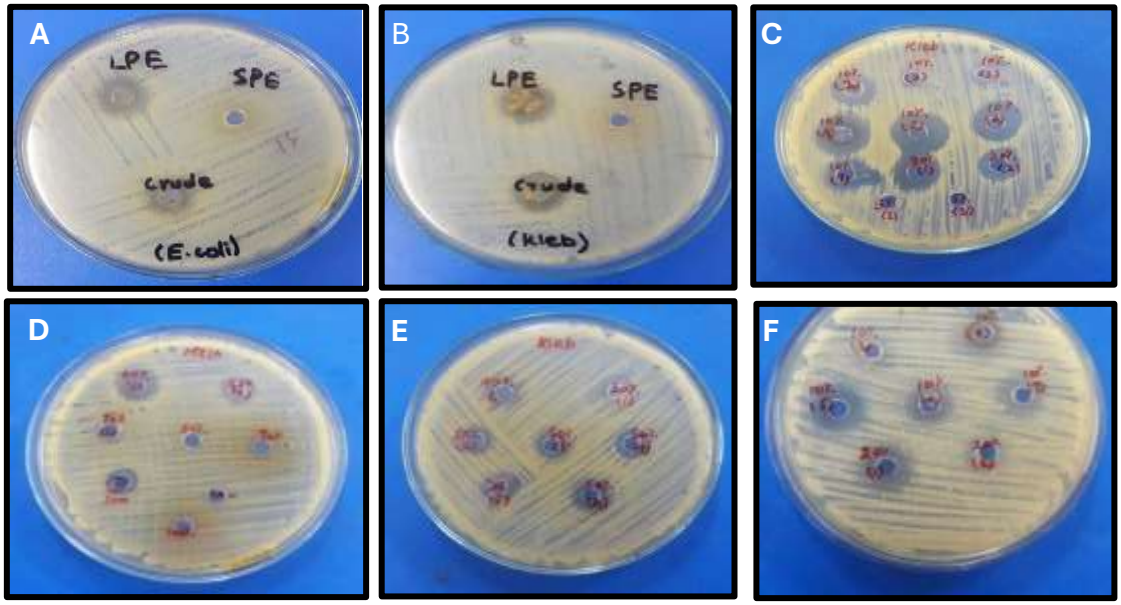

Figure 7. A: Antibacterial acrivity of crude extract, LPE and SPE of *Streptomyces* sp. PU-AK14 against *Klebsiella pneumoniae;* B: Antimicrobial acrivity of crude extract, LPE and SPE of *Streptomyces* sp. PU-AK14 against *Escherichia coli;*

C: Partially purified fractions against *Klebsiella pneumoniae;* D: Partially purified fractions against MRSA; E: Partially purified fractions against *Klebsiella pneumoniae;* F: Partially purified fractions against *Escherichia coli.*

Table 3: Total antioxidant potential of *Streptomyces* sp. PU-AK14

S. No.	Extract conc. (mg/ml)	Ascorbic acid (OD at 695nm)	Ascorbic acid equivalent level (mg/ml)		
			PU-AK14		
			Crude	LPE	SPE
1	0.025	0.22	0.0531	0.125022	-0.05755
2	0.05	1.353	0.112448	0.156708	-0.04548
3	0.1	2.166	0.160731	0.17934	0.091827
4	0.2	2.549	0.221588	0.222594	0.140613
5	0.4	3.542	0.320669	0.38052	0.164252
6	0.8	4.893	0.546996	0.823618	0.754211
7	1.6	5.356	0.939296	0.934267	1.727417
8	3.2	8.992	1.663542	1.48751	2.27714
9	6.4	10.343	1.990459	1.738985	2.94546

DISCUSSION

Actinobacteria produce a plethora of bioactive secondary metabolites that can be used for the treatment of human illness (Aftab *et al*., 2015). In this study, cultivation of *Streptomyces* sp. PU-AK14 was performed on a large scale to partially purify and screen its secondary metabolites. The resulting crude extract exhibited prominent antimicrobial activity against both gram-positive and gram-negative bacteria. The MIC against *Klebsiella pneumoniae* and *Escherichia coli* indicated a higher potency against gram-negative organisms compared to gram-positive organisms. Similarly, most of the purified fractions exhibited greater efficacy against gram negative organisms by agar well method. However, the microwell dilution method revealed that certain fractions exhibited higher activity against gram-positive organisms. This observation suggested that these specific compounds may be nonpolar in nature, leading to slower diffusion in the media. Amorim *et al*., (2020) also demonstrated the promising antimicrobial activity of *Streptomyces ansochromogenes* PB3 against the *Klebsiella pneumoniae* with 30mm zone of inhibition by well diffusion method. *Streptomyces ansochromogenes* PB3 was also active against gram positive bacteria. Another study by (Rammali *et al.,* 2024) also reported the isolation of *Streptomyces coeruleofuscus* YR-T from unexplored garden soil in Northwest Morocco. The strain exhibited significant activity against *Candida albicans, Escherichia coli, Staphylococcus aureus, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa*, as well as various other clinical MDR bacteria.

Artemia salina is an ideal marine organism for determining the cytotoxicity of crude extracts and partially purified compounds (Stalin *et al.,* 2022). The observed mortality percentage of up to 84.67% of *Artemia salina* induced by *Streptomyces* strain AK14 indicated the presence of cytotoxic compounds within the crude extract. The partially purified fractions, particularly AK143050F1S exhibited complete larval cell death at 100 mg/ml, while AK143050F2S exhibited 99.8% of larval cell death.

Streptomyces is known to produce a number of anti cancer substances such as bleomycin, dactinomycin, mitomycin, and doxorubicin (Aftab *et al*., 2015; Balitz *et al*., 1981; Bhattacharya and Mukherjee, 2015; Elkhateeb *et al*., 2020; Patel *et al*., 2010). The comparison of *Streptomyces* sp. PU-AK14's (crude extract) activity at various doses 12.5, 25, 50, and 100 mg/ml revealed an increase in antitumor activity with escalating extract concentrations. This trend was similarly observed for the fractions, where higher concentrations corresponded to increase antitumor effects. The *Streptomyces* strain PU-AK14 displayed cell mortality percentages of 66.789% via the MTT assay and 73.10% via the SRB assay, indicating its potential to generate compounds with potent antitumor activity. The retrieved fractions AK141111L, AK14541S, and AK1410(5-7)1L exhibited significant antitumor potential ranging from 67% to 80% that strongly suggested that these compounds possess inherent antitumor properties. Several other studies have reported the anticancer activity of crude extracts and pure compounds from *Streptomyces* species (Elkhateeb *et al.,* 2020; Fahmy and Abdel-Tawab, 2021). Another study by da Silva Lacerda *et al.,* (2018) purified L-asparaginase from *Streptomyces ansochromogenes* UFPEDA 3420 with significant *in-vitro* antitumor results in human PBMC, especially in T CD8+ lymphocyte subsets.

A study conducted by (Salehghamari *et al.,* 2024) investigated the microbial diversity of the Garmsar Salin River in Semnan province, Iran, an ecosystem that had not been previously explored. They discovered a notable isolate, *Streptomyces microflavus* M15, which exhibited significant cytotoxicity against human tumor cell lines while sparing normal cells ($P < 0.05$). Additionally, they observed selective activity against MCF7 cells.

Several actinobacterial genera are also a promising natural source of antioxidant compounds that are secure than synthetic antioxidants (Chandra *et al.,* 2020). The SPE and LPE derived from *Streptomyces* sp. PU-AK14 demonstrated equal percentage inhibition, indicating that both intra- and extracellular

compounds possess similar potential for antioxidant activity. In comparison to ascorbic acid (Standard) the crude extract did not exhibit significant results. However. some of the retrieved fractions AK143050F1S, AK143050F2S, AK145060F1S, AK145060F2S, AK1410(1-4)1L, AK1410(1-4)1S and AK1420(1-5)2S displayed prominent antioxidant activity, suggesting that these fractions contain compounds with significant antioxidant potential. The outcomes derived from the NO scavenging assay were consistent with those from the DPPH antioxidant assay. The total antioxidant potential assessed through the phosphomolybdenum reduction assay for both LPE and SPE demonstrated correlation of concentration and antioxidant activity. Several studies also reported the significance of antioxidative compounds produced by different *Streptomyces* species (Djebbah *et al*., 2022; Fahmy & Abdel-Tawab, 2021; Hosseini Abari *et al*., 2021). A study conducted by Hamed *et al*. (2024) documented the isolation of *Streptomyces* sp. strain RSE from a marine environment. They found that the extract of this strain contained 3,5-Dimethyl-1,3,4-hexanetriol, which exhibited significant antioxidant and antitumor activity.

CONCLUSION

The study reveals that partially purified fractions of *Streptomyces ansochromogenes* PU-AK14 is promising source of bioactive secondary compounds specifically antimicrobial, antitumor and antioxidant compounds. Altogether 18 fractions were retrieved with eight fractions bioactive against gram positive bacteria, five against gram negative bacteria, seven with promising antioxidant activity, four with substantial cytotoxicity against *Artemia salina* and three with significant antitumor activity against HCT 116 colorectal cancer cell lines. Moreover, the use of mass spectrometry and NMR spectroscopy facilitates the identification of these bioactive compounds, offering opportunities for their application in human medicine.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this manuscript

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