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Inhibitory Potential of Actinobacteria Isolated from Neelum Valley, Kashmir, Pakistan against Typhoidal *Salmonella*

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ABSTRACT: *The emerging antimicrobial resistance (AMR), and finding of new antimicrobials and chemotherapeutics is a need of the time. In this study, N=32 actinobacteria strains were recovered from the soil of Neelum Valley, Kashmir, Pakistan, to screen their inhibitory activity and metabolomic potential against MDR typhoidal Salmonella. The TLC pattern and HPLC-UV-MS analysis of the extracts of isolated strains showed the presence of a variety of bioactive compounds. The actinobacteria strains B6, D13, B11.2 and others were found to be the most prolific producers of diverse secondary metabolites. In the screening for inhibitory activity against MDR typhoidal Salmonella, the extracts of strains B6, B16, B21 and D13 were found to be the most active exhibiting up to 20 mm zone of inhibition. Similarly, the cytotoxicity potential of the extracts was measured against Artemia salina by determining % larval mortality. The extract of strain Kel 1311A showed 91% mortality followed by the extracts of strains AzB1, kel 1391, B13, B8, and B4 which showed % mortality up to 87.3%. 90%, 88% and 85.41% respectively. The HPLC-UV/MS analysis revealed the presence of a variety of compounds with molecular weights of 400, 510 and 519 Daltons in the extract of strain B4 and 651, 356, 378 and 400 Daltons in the extracts of strain D13. The study suggested that actinobacteria living in the soil of Neelum Valley Kashmir are a rich source of novel bio-active, cytotoxic and anti-microbial compounds.*

Keyword: Actinobacteria, MDR, Salmonella, Antibiotics, Cytotoxicity, HPLC-UV-MS

INTRODUCTION

Actinobacteria are prokaryotes which belong to the Gram-positive subdivision of bacteria (Istianto et al., 2012) and are abundantly found in soil and freshwater. Actinobacteria are the top producers of antibiotics and other pharmaceutically significant compounds. Actinobacteria are distinct in terms of their genomes as they have generally higher GC contents in their DNA (>55%) as well and they have a characteristic morphology that bears a resemblance to both bacteria and fungi (Rai et al., 2018; Ghai et al., 2012; 2013). One of the famous features of actinobacteria is their tremendous potential of producing various bioactive compounds ranging from antibiotics, and anti-tumour to agro-active compounds as well as plant growth hormones (Passari et al., 2015). As per reports, about 70% of the known antibiotics in the last five decades are of actinobacterial origin. While overall about 80% of the known antibiotics are discovered from actinobacteria, predominantly from the genera of *Streptomyces* and *Micromonospora* (Jensen et al., 1996). *Salmonella* spp. is a very well-known pathogen generally causing typhoid and enteric fever and belongs to the enterobacteria group. The *Salmonella* spp. are readily transmitted through water, food and animals derived products and are a major cause of concern due to their capability of acquiring multi-drug resistant (MDR) and XDR (Extensively- drug-resistant) status (Sánchez et al., 2011). Recently there has been a general increase in the number of multi-drug resistant (MDR) and XDR (Extensively- drug-resistant) bacterial infections, consequently, there is a general requirement to produce and find new anti-microbials to counter the resistant pathogens and microbes (Parisi et al., 2018; Dancer et al., 2004). Actinobacteria are undoubtedly the most reliable and natural resource for finding new antibiotics and other related compounds (Hassan et al., 2011).

The toxic nature of potent antibiotics has prompted their restricted use, but numerous anti-infectious agents have been endorsed to date as having considerable cytotoxicity. To escape this situation, search for the most recent anti-infection agents that are more powerful and have no toxic side effects. Actinobacteria from underexplored environments have always been associated with potent and often novel antimicrobials and other related compounds (Nawaz et al., 2023). Among the underexplored environments, mountains and remote valleys have always been of

considerable importance as these habitats provide a particular climatic condition with a variety of nutrients and their peculiar weather may also be a factor in the selective expression of different biosynthetic gene clusters (Warnecke et al., 2005; Tang et al., 2016; He et al., 2023). In Neelum Valley, the mean annual temperature ranges from -10.9 to 17.3 °C while the mean annual precipitation is between 246-1048 mm (Zubair et al., 2019). These conditions generally suits actinobacterial growth as they normally grow at low temperatures and Neelum valley provides an excellent site for the harboring of actinobacteria. This study was designed to screen bioactive secondary metabolites of actinobacteria isolated from the Neelum Valley Kashmir (34°35'54.52"N, 73°54'26.27"E) against MDR (Multi drugs resistant) *Salmonella* and to gain insights into their biochemical profiles. (MDR) Salmonella as isolates are defined as MDR if they are resistant to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole, and as XDR if they are MDR, non-susceptible to fluoroquinolones, and resistant to third-generation cephalosporins (Chatham et al., 2019).

MATERIALS AND METHODS

Sample Collection and Processing

Humus-rich soil samples were collected from Neelum Valley Kashmir, Pakistan. Samples were dug from 20cm depth and 3km away from each other. Samples were kept in sterile sampling bags and were transferred to the labs under aseptic conditions.

The samples were subjected to dry heat for 1 hour in a hot air oven at 120 $\mathring{C}$ to selectively isolate actinobacteria isolates (Fang et al., 2017).

In a test tube containing 9ml sterile water, 1g of collected sample was added. The sample was transferred to another 9ml sterile water tube and the step was repeated ten-fold down to 10^{-4} (Hayakawa et al., 2018).

Selective Isolation and Pure Cultures of Actinobacteria

The 100µl pretreated 10^{-3} and 10^{-4} dilutions were taken and spread on Starch Casein KNO_3agar, pH 7.2 to 7.4 at 28° C for 2-3 weeks. Colonies were picked from the crowding and were further subcultured on GYM and A media (Shirling and Gottlieb, 1996) to obtain the pure cultures of the isolated strains.

Taxonomic Characterization of the Selected Actinobacteria

In the case of morphological identifications various features were observed such as the colour of aerial mycelium, the colour of substrate mycelium, and the colour of pigment (colony shape, size, consistency, elevation and margin) (Adegboye et al., 2016). Different biochemical tests like melanin formation, utilization of sugar, utilization of organic acid, utilization of oxalate, hydrolysis of esculin and solvent extraction were performed. Melanin formation and utilization of different sugars were tested.

Determination of Antimicrobial Activity of the Selected Actinobacteria against Typhoidal *Salmonella*

Preparation of Crude Extracts

A 10 mL of Starch Casein KNO_3 broth was inoculated with isolates and were kept at 28 C in a shaking orbital incubator for 3 days. This step was followed by another inoculation of 50 mL of SC broth with 2 mL of enriched culture and the flasks containing fresh inoculates were again re-incubated at the same conditions for 10 days. After the incubation period, 1% of Amberlite XAD-16 N resin was added, to the fermented broth which was then sonicated for 25 m at 30 KHz to recover attached intracellular metabolites. This sonicated mixture was then placed for agitation on an orbital shaker overnight after which the cellular mass was disposed of through centrifugation by separating the broth containing the metabolites. Finally, methanol was used for the elution of the metabolites attached to XAD resin (Nawaz et al., 2023). Secondary screening of the active strains consisted of subjecting extracted metabolites against some multi-drug resistance (MDR) bacterial strains (Singh et al., 2014).

Chemical Screening of Selected Crude Extracts

TLC Analysis of Crude Extracts

The active fractions were further analyzed by TLC and High-performance liquid chromatography (LC-MS) using reverse phase silica column (Wu et al., 1984). HPLC grade methanol was used to dissolve the extracted methanol, at the ratio of 5mG/mL.TLC plates (20 × 20 cm Merck-Silica with Aluminium base, thickness (20 µm) with binder Polymeric fluorescent indicator) were used for TLC analysis. The optimum solvent system was chosen based on repeated trials and it was found to be Chloroform: methanol (3:1) (Nawaz et al., 2023).

For LC-MS analysis samples were prepared by dissolving the extracts in 500 µl methanol. Furthermore, this methanolic mixture was 5 times diluted in 50% acetonitrile. The diluted samples were then filtered through 2µm pore-size disposable syringe filters (Sartorius Minisart SRP-15 syringe filters) and then centrifuged at 14000 g to remove any particles. Samples were then transferred to special LC-MS glass vials which were further placed in the sample tray of the UPLC-MS chromatography system (Waters Acquity UPLC-MS System).

Brine Shrimp (*Artemiaselena*) Microwell Cytotoxicity Assay

The 1 gram of *Artemiaselena* dried eggs were added to artificial seawater in a 400 ml liquid tank while keeping the surrounding environment dark. The tanks were aired by bubbling air and left for 2-3 days at ambient temperature. The phototrophic larvae were collected and added into each well of the microwell plate, while extracts dissolved in DMSO were tested at a concentration of mg/mL, while 20 µl DMSO was added as the negative control and Actinomycin-D as the positive control.

By using the following formula, the mortality rate 'M' was calculated as;

$$M = \frac{A - B - N}{G - N} \times 100$$

Where:

A = several dead larvae after 24h

B = No. of dead larvae in a blank sample after 24h

G = Total no. of M = total dead larvae after 24h

N = No. of dead larvae before performing the test larvae (Sajid et al., 2011)

RESULTS

Taxonomic Characteristics of the Selected Actinobacteria Strains

The polyphasic taxonomic approach was adopted including, morphological, physiological and biochemical identification-based characterization of the strains. The strains were analyzed for colony morphology, elevations, margins (Table 1), and gram staining profile (Fig. 1, 2). The biochemical identifications included sugar utilization profile, esculin hydrolysis melanin formation etc (Table 2). A total (N=32) different biologically active strains of actinobacteria were isolated from humus-rich soil from Neelum Valley Kashmir. The strains were isolated and purified on GYM and SCA medium and were kept at 28ºC (for 10-14 days) followed by biological and chemical characterization. The strains were

named as B2, B4, B6,B7,B8, B10,B11, B13, B14, B16, B17, B18, B11-2, B21, D1, D3, D4, D5, D6, D7, D9, D10, D13, D15, Kel 1391, Kel 1392, Kel 1393, kel 1311A, Kel 1311B, Kel 1312, Azk28, AzB1. In the case of melanin formation, Kel 1391, Kel 1392, Kel 1393, kel 1311A, Kel 1311B, Kel 1312, Azk28, and AzB1 were found to be strongly positive. In the case of sugar utilization, all strains were positive for sucrose except B16 and D7. In the case of mannose, all strains were positive except B11, D6 and kel 1392, similarly in the case of fructose, all strains except Kel 1392, B10 and D15 were positive. The maltose test came up with all strains being positive except B16 and D7. For the use of xylose, all strains were positive except B7, D1 and Azk2.3. For lactose use, all strains were positive except D1 and D9. For the use of Arabinose, all strains were positive except B4, D4, D10 and AzB1 (Table 2).

Antibacterial Activity of the Extracts

Based on the primary broth cultures screening results, the 32 isolates were subjected to extraction of crude extracts and the crude extracts were analyzed for their antibacterial potential using a variety of MDR *Salmonella* as target strains along with *S. aureus* and *E. coli* as the representative Gram-positive and Gram-negative strains, by the disc diffusion method. Among all the isolates the strains B6, B11.2 and B21 were found to be the most active showing significant zones of inhibition against *S. aureus*, *E. coli* and MDR strains of *Salmonella*. The extracts of B6 isolates showed inhibitory zones of 20 mm, 24 mm and 20 mm against *S. aureus*, *E. coli* and MDR *Salmonella* respectively. Similarly isolate B11.2 showed significant zones of inhibition of 13 mm, 25 mm, 19 mm and 13 mm against *S. aureus*, *E. coli*, MDR4 and MDR5 of *Salmonella* respectively (Fig. 3). Likewise, isolate S21 showed a considerable zone of inhibitions of 15 mm, 13 mm and 13 mm against *S. aureus*, *E. coli* and MDR strain 5 of *Salmonella* (Table 3).

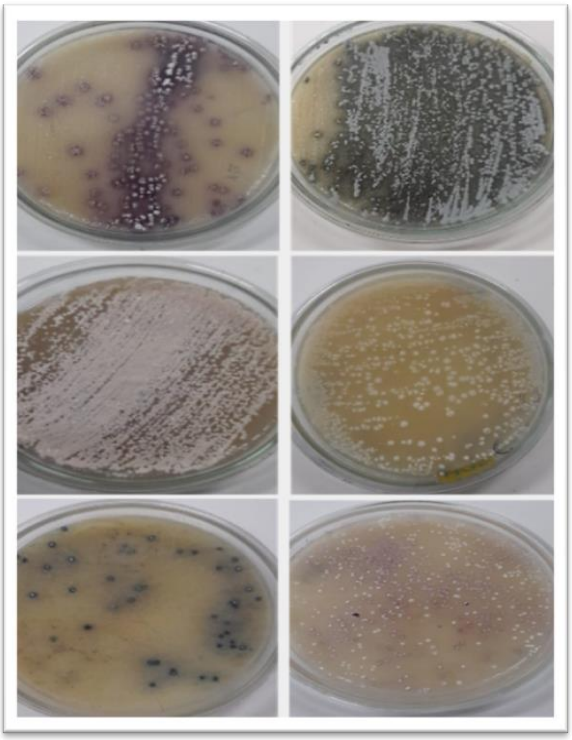

Fig. 1: Morphological appearance of the actinobacteria strains isolated from Neelum Valley Kahsmir, Pakistan

Table 1: Morphological characteristics of actinobacteria isolated from Neelum Valley Kahsmir, Pakistan

	Size of Colony	Colour of Substrate Mycelium	Colour of Aerial Mycelium	Pigmentation	Colony Margins	Colony Shape	Colony Texture
B2	Small	White	White	White	Smooth	Round	Hard
B4	Moderate	White	White	White	Rhizoid	Round	Hard
B6	Small	White	White	White	Entire	Round	Hard
B7	Moderate	Cream	White	Cream	Lobate	Flat	Hard
B8	Large	Yellow	White	Yellow	Erose	Round	Hard
B10	Moderate	Greyish Black	White	Greyish Black	Smooth	Round	Hard
B11	Small	Dark Yellow	Grey	Grey	Rhizoid	Round	Hard
B13	Large	Yellow	Light Yellow	Yellow	Entire	Round	Powdery

B14	Large	Black	Pinkish White	Black	Lobate	Round	Hard
B16	Moderate	Black	Brownish Grey	Black	Erose	Round	Hard
B17	Small	Brown	Whitish Grey	Brown	Smooth	Round	Soft
B18	Small	Pink	White	Pink	Rhizoid	Irregular	Hard
B11.2	Moderate	Brown	White	Orange	Entire	Round	Hard
B21	Large	White	Skin	White	Lobate	Round	Hard
K4.1	Small	Purple	Grey	Purple	Erose	Round	Soft
D1	Moderate	Purple	White	Purple	Undolate	Irregular	Powdery
D3	Large	Whitish Brown	Pink	Brown	Entire	Round	Hard
D4	Large	Off White	White	Pink	Lobate	Round	Hard
D5	Small	Yellow	Cream	Yellow	Erose	Round	Powdery
D6	Moderate	Black	Yellow	Black	Undolate	Flat	Soft
D7	Large	White	White	White	Entire	Irregular	Hard
D9	Small	White	White	Black	Lobate	Round	Hard
D10	Small	Yellow	White	Yellow	Erose	Round	Hard
D13	Moderate	Purple	Cream	White	Undolate	Round	Hard
D15	Moderate	White	Black	White	Smooth	Round	Hard
Kel. 1391	Small	Purple	Grey	White	Rhizoid	Round	Hard
Kel. 1392	Large	White	White	Cream	Entire	Round	Hard
Kel. 1393	Small	Black	White	Yellow	Lobate	Round	Hard
Kel. 1311A	Small	White	Skin	Greyish Black	Erose	Irregular	Hard
Kel. 1311B	Large	Grey	Cream	Dark Yellow	Undolate	Round	Hard
Azk 2.3	Moderate	Yellow	White	Yellow	Undolate	Round	Hard

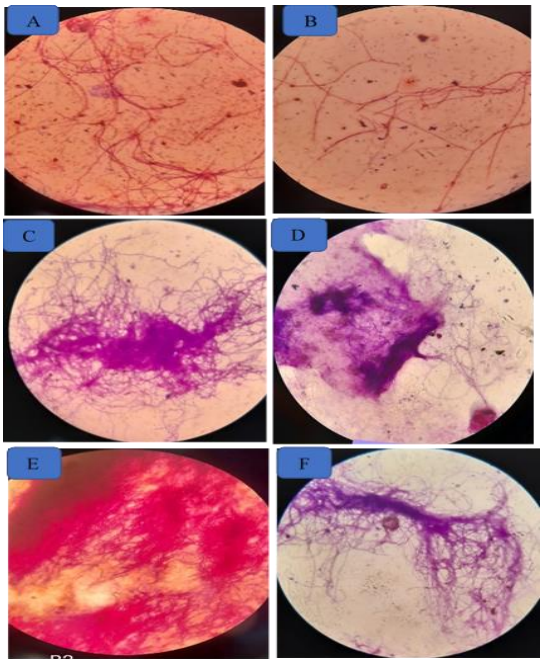

Fig. 2: Isolated actinobacterial strains showing filamentous appearance after Gram staining, under the microscope (10X).

Table 2: Biochemical characteristics of the selected actinobacteria strains isolated from Neelum Valley Kashmir

Actino bacteria strains	Melanin	Utilization Of Sugars						
		Glucose	Xylose	Maltose	Lactose	Sucrose	Fructose	Arabinose
B2	+	+	+	+	+	+	+	+
B4	+	+	+	+	+	+	+	-
B6	+	+	+	+	+	+	+	+
B7	+	+	-	+	+	+	+	+
B8	+	+	+	+	+	+	+	+
B10	+	+	+	+	+	+	-	+
B11	+	+	+	-	+	+	+	+
B13	+	+	+	+	+	+	+	+
B14	+	+	+	+	+	+	+	+
B16	+	+	+	+	+	-	+	+
B17	-	+	+	+	+	+	+	+
B18	+	+	+	+	+	+	+	+
B11.2	+	+	+	+	+	+	+	+
B21	+	-	+	+	+	+	+	+
K4.1	+	+	+	+	+	+	+	+

D1	+	+	-	+	-	+	+	+
D3	+	+	+	+	+	+	+	+
D4	+	+	+	+	+	+	+	-
D5	-	+	+	+	+	+	+	+
D6	+	+	+	-	+	+	+	+
D7	+	+	+	+	+	-	+	+
D9	+	+	+	+	+	+	+	+
D10	+	+	+	+	-	+	+	-
D13	+	+	+	+	+	+	+	+
D15	-	+	+	+	+	+	+	+
Kel.1391	+	+	+	+	+	+	+	+
Kel.1392	+	+	+	-	+	+	-	+
Kel.1393	+	+	+	+	+	+	+	+
Kel. 1311A	+	-	+	+	+	+	+	+
Kel. 1311B	+	+	+	+	+	+	+	+
Azk2.3	+	+	-	+	+	+	+	-
AzB1	+	+	+	+	-	+	+	+

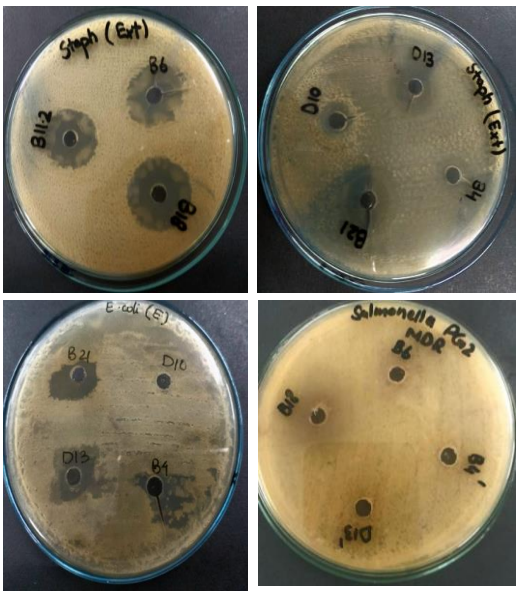

Fig. 3: Inhibitory activity of the extracts of the isolated actinobacteria strains against the MDR *Salmonella* and selected pathogenic strains

Table 3: Antimicrobial activity and cytotoxicity of the methanolic extracts of the isolated actinobacteria strains

Actino bacteria Extracts	Zone of **Inhibition (mm)**							
	E. coli	***S. aureus***	***Salmonella* MDR Strain 1**	***Salmonella* MDR strain 2**	***Salmonella* MDR strain 3**	***Salmonella* MDR strain 4**	***Salmonella* MDR strain 5**	**Percentage larval mortality**
B2	12	14	7	-	10	-	-	75
B4	7	10	-	-	-	-	9	88
B6	20	24	-	-	-	-	20	74
B7	11	15	-	-	-	9	12	77
B8	13	11	-	14	-	-	7	83
B10	12	14	-	-	-	-	-	56
B11	10	-	-	-	9	-	9	65
B13	14	20	-	18	-	15	-	85
B14	-	-	14	10	-	-	-	76
B16	13	25	13	9	10	19	13	57
B17	-	-	-	-	-	-	-	47
B18	-	25	-	10	-	20	19	60
B11.2	13	25	17	-	-	13	19	79
B21	15	13	-	-	13	-	20	38
K4.1	10	-	7	10	14	12	14	70
D1	12	10	-	-	-	-	-	55
D3	-	-	13	-	12	10	7	49
D4	-	12	-	-	-	-	-	10
D5	10	15	-	-	-	16	12	30
D6	8	-	7	9	-	-	12	58
D7	11	-	14	-	13	16	14	65
D9	-	-	13	-	-	14	-	37
D10	7	11	-	-	10	-	-	45
D13	17	15	-	7	-	19	20	20
D15	12	19	-	-	-	-	-	15
Kel.1391	12	14	-	-	20	-	15	90
Kel.1392	10	9	12	-	-	12	-	55
Kel.1393	-	-	-	20	-	-	-	41
Kel.1311A	7	10	9	-	10	13	14	91
Kel.1311B	12	20	-	21	-	-	10	69
Azk2.3	9	7	-	-	7	-	9	25
AzB1	7	14	7	20	12	15	8	87

Cytotoxicity of the Extracts against *Artemia salina*

The most noteworthy larval mortality was shown by the extracts of strain kel 1311A of 91%, trailed by AzB1, kel 1391, B13, B8, and B4 showed a percentage mortality rate of 87.3%. 90%, 88% and 85.41% separately. B2, B6, B7, B14, B11.2 and K4.1 had mortality rate over 70% that is 75%, 74%, 77.2%, 79% and 70.83% separately. While B11, B18, and kel 1311B had rate mortality of over 60% that is 65%, 60%, 60%, and 69% respectively (Table 3).

Metabolomics Profile of the Extracts

The metabolomic profile of the extracts was assessed by subjecting the extracts to chemical analysis, such as Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC)-MS.

TLC Analysis of the Selected Extracts

The silica plates used for TLC were observed under UV 254 and 366 nm and it was found that Kel 1311B, Kel 1312, Azk28, and AzB1 produced distinct band patterns under UV (Fig. 4). However alternative staining with Anisaldehyde yields various coloured spots for the extracts of strains B11.2, B18, D13, D15, B18, D3, D5, and D1 suggesting that they are potent producers of various compounds.

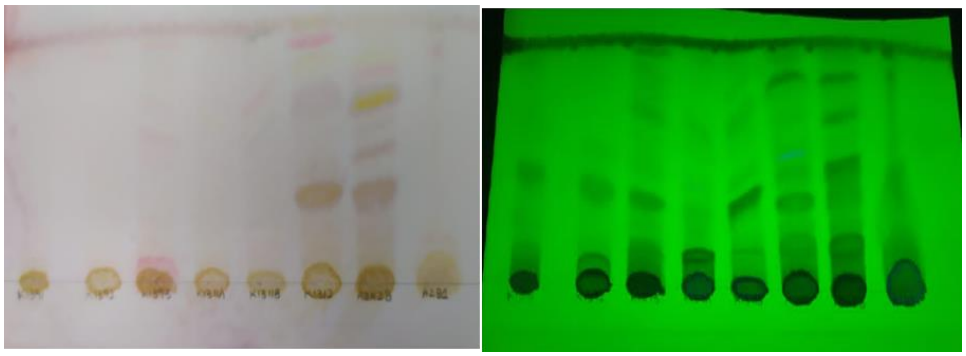

Fig. 4: TLC Pattern of the selected metabolites derived from actinobacteria

HPLC-UV- MS Analysis

The methanolic extracts of two strains including, D13 and B4 were processed for HPLC- UV-MS analysis, which produced distinct peaks at various retention times (t_R) with molecular masses in the range of 300-500 Daltons. The molecular masses calculated for some of the selected compounds were 519, 563, 607, 356, 400, 510 and 519 in

the extract of strain B4 (Fig. 5). While in the case of the extract of strain D13 compounds with molecular weights of 563,334, 607, 354, 651, 356, 378 and 400 were detected (Table 4).

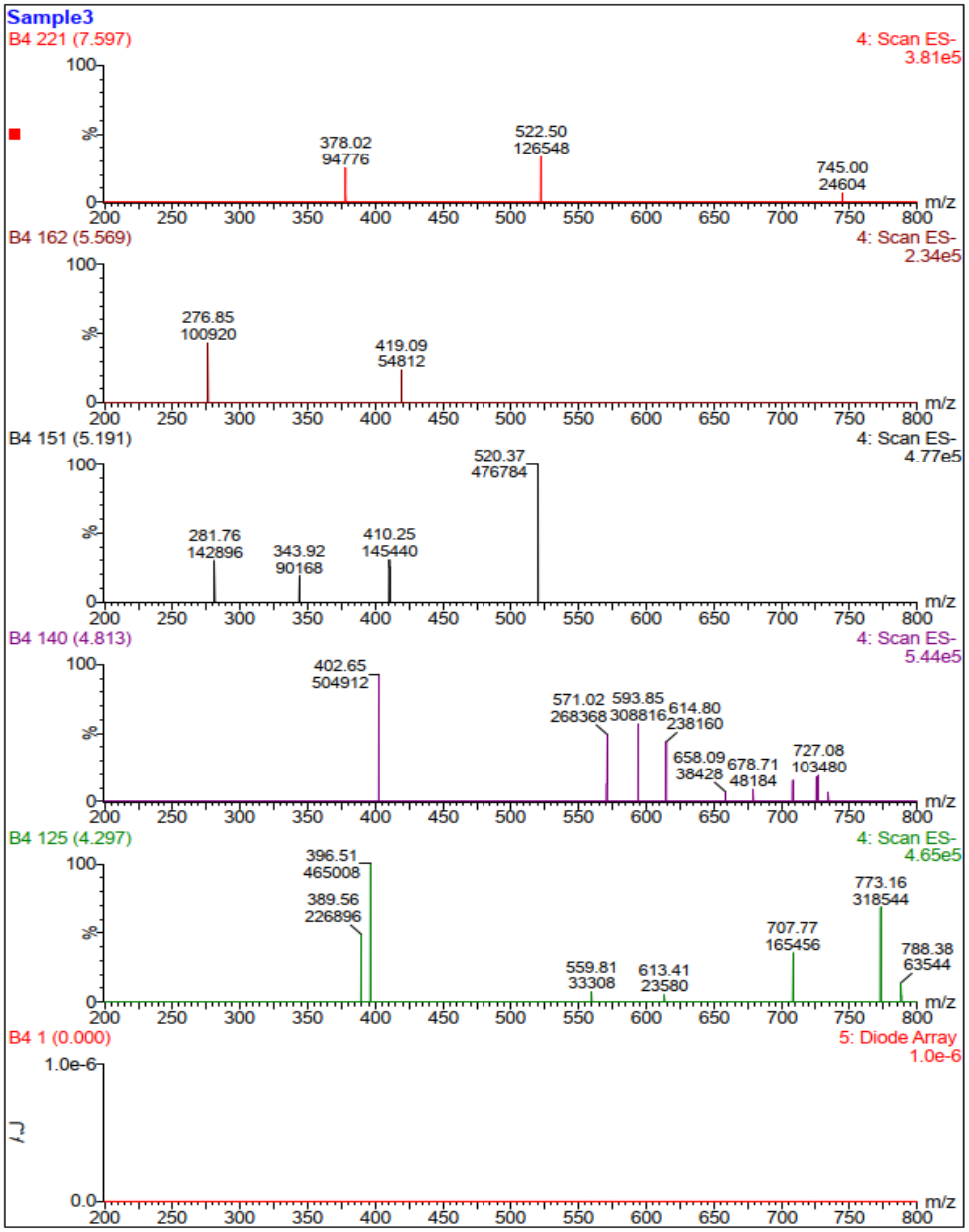

Fig. 5: HPLC-ESI/MS spectra of the methanolic extract of *Streptomyces* sp. B4

Table 4: HPLC-MS analysis and metabolomics profiling of bioactive compounds produced b//y the selected actinobacteria strains

Strains	Thin Layer Chromatography(TLC) profile			HPLC retention time (t_R/min)	(+) and (-)-ESI-MS:(m/z)	Molecular weights (Da)	Bioactivity in (mm) against MDR *Salmonella*
	UV Visualization		Staining with Anisaldehyde/H_2SO_4				
	254 nm	366 nm					
B4	4 band	3 band	Pink bands appear	4.179	520.38 $[M + H]^+$	519	9mm zone of inhibition against MDR *Salmonella*
				4.230	564.36 $[M + H]^+$	563	
				4.289	608.34 $[M + H]^+$	607	
				4.374	357.32 $[M + H]^+$	356	
				4.417	401.89 $[M + H]^+$	400	
				4.570	511.36 $[M + H]^+$	510	
D13	4 band	5 band	Purple bands appear	4.187	520.38 $[M + H]^+$	519	10mm zone of inhibition against MDR *Salmonella*
					564.29 $[M + H]^+$	563	
				4.238	564.36 $[M + H]^+$	563	
				4.289	335.30 $[M + H]^+$	334	
					608.27 $[M + H]^+$	607	
				4.323	355.30 $[M + H]^+$	354	
					652.47 $[M + H]^+$	651	
				4.366	357.25 $[M + H]^+$	356	
				4.400	379.28 $[M + H]^+$	378	
				4.434	401.30 $[M + H]^+$	400	

DISCUSSION

Various studies suggest that northern areas and other less explored regions of Pakistan such as Galliyat, Himalayas and Gilgit Baltistan as well as the Cholistan deserts in the central and southern regions have a tremendous potential to produce a variety of phytochemicals and other bioactive compounds (Ishaque et al., 2021; Liu et al., 2020; Cheema et al., 2020). N=32 strains of soil actinobacteria from the Neelum Valley of Kashmir were selected. The morphological, physiological and biochemical features suggested that predominant of these belong to the genus *Streptomyces* of the actinobacteria (Nomi, 1960; Taddei et al., 2006).

Among all studied sugars, glucose was efficiently utilized by all strains however, mannose and mannitol were also greatly utilized by the isolates. Arabinose was relatively least consumed by the isolates. Melanin formation test can be a good tool to find out the potential actinobacteria isolates, nearly all of the isolates produced melanin to some extent with various intensities

(Manivasagan et al., 2013; Kamarudheen et al., 2019).

Salmonella typhi resistant to chloramphenicol, ampicillin, and trimethoprim (i.e., multidrug-resistant [MDR] strains) have been responsible for numerous outbreaks in countries in the Indian subcontinent, Southeast Asia, and Africa. The isolates were screened against MDR *Salmonella*, and among all the isolates B6, B11.2 and B21 were found to be the most active isolates showing significant zones of inhibition against *S. aureus*, *E. coli* and MDR strains of *Salmonella* suggesting that these extracts can be a good source of producing potent antimicrobials against these difficult to treat pathogenic strains of *Salmonella* (Rowe et al., 1997; Alanine et al., 2007). Although previous studies on the other side of Kashmir showed a variety of antimicrobial-producing actinomycetes from the region having antimicrobial potential these studies are limited to conventional pathogens such as *S. aureus* as well as *M. tuberculosis* and the best of our knowledge none of these include MDR or XDR pathogens (Shah et al., 2017).

TLC has traditionally been utilized to screen the prolific compounds producing actinobacteria and in this study, the isolates kel.1311A, AzB1, Kel.1391, Kel.1392 B11.2, B18, D13, D15, B18, D3 and D5 produced a variety of bands when visualized under 254 and 366 nm UV light. The same extracts also produced different coloured bands when alternatively stained with anisaldehyde (Balagurunathan et al., 2020; Wang et al., 2016).

For the cytotoxic assay, *Artemia salina* larvae were used and significant mortality rates were observed for the extracts of kel. 1391 and azB1 showed 90% and 87% mortality rates respectively. The rest of the isolates had also a very significant cytotoxic potential which depicts that these isolates can considered good producers of cytotoxic compounds (Nafis et al., 2018).

Most of the antibiotics and bioactive compounds have an average size of molecular weights ranging from 300 to 1000. Generally, anything below 1000 can be considered interesting in terms of antibiotic discovery. In this study, the molecular weights for the extracts analyzed on HPLC-UV-MS were found to be below 1000 such as as 400, 510 and 519 for B4 and 651, 356, 378 and 400 for the extracts of D13 suggesting that there can be possible antimicrobials within the extracts of these isolates as suggested by the antimicrobial and

cytotoxic assays as well (Cooper et al., 2018).

CONCLUSION

The promising results of the extracts of the isolated strains in terms of cytotoxicity, antimicrobial assays and the interesting range of molecular weights of the extracted compounds conclude that Neelum Valley, Kashmir, Pakistan is a rich habitat of interesting actinobacteria, which can produce fascinating bioactive compounds. The study can further be extended by increasing the sample size and actual purification of the bioactive compounds aided by modern bioinformatics tools such as whole genome sequencing-based genome mining MS spectrometry-based molecular networking, and structure elucidation of the pure compounds.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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