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Antimicrobial Potential of Actinomycetes Isolated from Soil of Nepal

**Krishna Prasad Pant^{1,2*}, Madan Singh Bohara^{1,2}, Yogendra Shah³, Amrit Acharya^{1,4},
 Supriya Sharma¹ and Binod Lekhak¹**

¹Central Department of Microbiology, Tribhuvan University, Nepal

²Central Department of General Science, Far Western University, Nepal

³Kathmandu College of Science and Technology, Nepal

⁴Pulchowk Campus, Institute of Engineering, Tribhuvan University, Nepal

*Corresponding author: krishnapant@fwu.edu.np

Abstract

Antimicrobial resistance (AMR) to antibiotics is increasing rapidly, which is a serious public health problem worldwide, so the issue of AMR should be addressed in time. Many studies have been conducted in Nepal to isolate potent Actinomycetes strain from soil and water in various parts of the country. The aim of this review is to find novel Actinomycetes isolates from Nepal producing bioactive compounds capable of suppressing the growth of multidrug-resistant pathogens. Preliminary screening in these studies in Nepal have identified isolates with notable antimicrobial activity against various pathogenic bacteria including *Escherichia coli*, ESBL *E. coli*, *Staphylococcus*, MRSA, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Salmonella Typhi* and others. Findings from several studies in Nepal indicate that Actinomycetes isolated from different habitats of Nepal can produce a wide array of bioactive compounds like diketopiperazines, actinomycins, bacterial alkaloids, anthramycin-type antibiotics, lipase inhibitors,

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cytotoxic metabolites, antifungal and antitumor antibiotics. In conclusion various regions across Nepal contain novel Actinomycetes strains that can produce novel bioactive compounds and effective antimicrobial drugs to combat the growing problem of AMR.

Keywords : Actinomycetes, antimicrobial resistance (AMR), bioactive compounds

Introduction

The pathogenic and multi-drug resistant (MDR) microorganisms are a great threat to humans because they are rapidly developing resistance against many antibiotics making treatment difficult, causing a serious public health problem globally (Lekhak et al., 2018; Pandey et al., 2021; Sapkota et al., 2020; Thapa, et al., 2024b; Yadav et al., 2024b). The WHO recognizes antimicrobial resistance (AMR) as a global health crisis, resulting in several fatalities each year from multidrug-resistant illnesses if not adequately managed (Thapa, et al., 2024a). Thus, there is an immediate demand to search for novel medicines and alternative antimicrobial agents to treat infections by multidrug-resistant pathogens.

Actinomycetes are filamentous, Gram-positive bacteria that can produce natural antibiotics and other bioactive substances (Aryal, et al., 2025a; Bajagain & Limbu, 2024; Bajracharya et al., 2024; Bhandari et al., 2022; Bhattarai et al., 2022; Budhathoki & Shrestha, 2020; Luitel et al., 2021; Sapkota et al., 2020; Shrestha et al., 2021). They are mainly isolated from soil making them valuable in various fields including agriculture, industry, and pharmaceuticals throughout the world (Sapkota et al., 2020). Approximately 45% of bioactive compounds obtained from microbes derived from Actinomycetes, with the genus *Streptomyces* responsible for the being responsible for about 70% production of total antibiotics production (Shrestha et al., 2021).

Due to Nepal's distinct temperature and varied topography, a variety of microbes, including Actinomycetes, can survive there and may produce bioactive and antimicrobial substances (Gurung & Rai, 2021; Sah et al., 2021; Sapkota et al., 2020; Shrestha et al., 2021; Thapa, et al., 2024b). However, many research works from Nepal has reported different strains of Actinomycetes producing novel bioactive compounds effective against multidrug-resistant pathogens (Lekhak et al., 2018). This review was done to find the potential Actinomycetes isolates and their bioactive compounds that were effective against multidrug pathogens. These type of studies are conducted continuously to identify the novel Actinomycetes strains producing novel antimicrobial compounds that can combat the problem of AMR. The objectives of the study were:

- To review studies identifying various types of Actinomycetes isolated from different regions of Nepal.

- To review the characterization of Actinomycetes isolates exhibiting antimicrobial activity.
- To review the characterization and identification of bioactive compounds produced by Actinomycetes isolates.

Materials and Methods

Search Strategy and Eligibility Criteria

A thorough literature search was performed utilizing two electronic databases: PubMed and Google Scholar. The inquiry relied exclusively on original research papers published in English from 2018 to 2025. The review focused exclusively on studies of soil-derived Actinomycetes in Nepal.

Inclusion Criteria

Research publications presenting primary research and original work in the English language during the period 2018–2025 were considered; articles reporting studies on the isolation of Actinomycetes from soil samples collected in Nepal were included in the analysis.

Exclusion Criteria

Review articles (systematic reviews and meta-analyses) as well as those dealing with Actinomycetes isolated from places other than Nepal would be excluded.

Data Extraction

Important variables including the number of isolates, identified Actinomycetes, identification techniques, detection methods, activity against Gram-positive bacteria, activity against fungi, activity against Gram-negative bacteria, anticancer activity, compound identification, and the method of compound identification were collected. Additional details such as the Author(s), DOI/PMID, title, sample type, sampling site, elevation (m), and study period were also collected.

Results

We identified a total of 37 studies conducted in Nepal, of which 5 were PubMed and 32 from google scholar. Out of these 23 studies were selected for the review based on the inclusion criteria. These studies were conducted in different parts of Nepal such as terai, hills and high-altitude regions. Actinomycetes were mainly grown on starch casein agar (SCA) media with few studies used Actinomycetes isolation agar (AIA) media, ISP-2 and ISP-4 media. A total of 679 Actinomycetes isolates were isolated from soil and water samples at different altitudes of Nepal. Among these isolates 592 (87.19%) were identified by phenotypic and biochemical methods while the remaining 87 (12.81%) were identified by molecular methods given in table 1 (Bhandari et al., 2022; Budhathoki & Shrestha, 2020; Gurung & Rai, 2021; Karki & Magar, 2024; Lekhak et al., 2018; Sapkota

et al., 2020; Shrestha et al., 2021).

Table 1

Number of Actinomycetes isolates by different methods

Methods of Identification	No of Isolates
Phenotypic methods and Biochemical methods	592 (87.19%)
Molecular methods	87 (12.81%)
Total	679

In this review 679 Actinomycetes isolates were identified using different methods. Among 592 Actinomycetes isolates identified by phenotypic methods *Streptomyces* (36) was the predominant genus followed by *Nocardia* (17), *Micromonospora* (16), while 523 remained unidentified at the genus level. Among 87 isolates identified by molecular methods 82 were identified as *Streptomyces* (82) followed by *Nocardia* (2), *Nocardiopsis* (1), *Amycolatopsis* (1) and *Nocardia nepalensis* sp. nov., (1) the latter being a novel strain isolated from soil of Sarangkot, Kaski at an altitude of 1378 m (chart 2) Aryal et al., 2024b). Common identified genera included *Streptomyces*, by both phenotypic and molecular methods as shown in chart 2, 3 respectively. *Streptomyces* was the most abundant genus found in these studies (Sah & Dhakal, 2023; Sapkota et al., 2020; Shrestha et al., 2021).

Chart 2

Methods of identification and Identified Actinomycetes

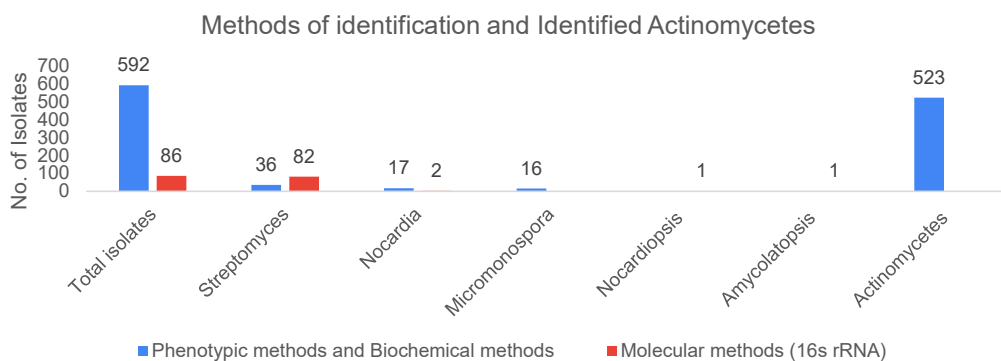
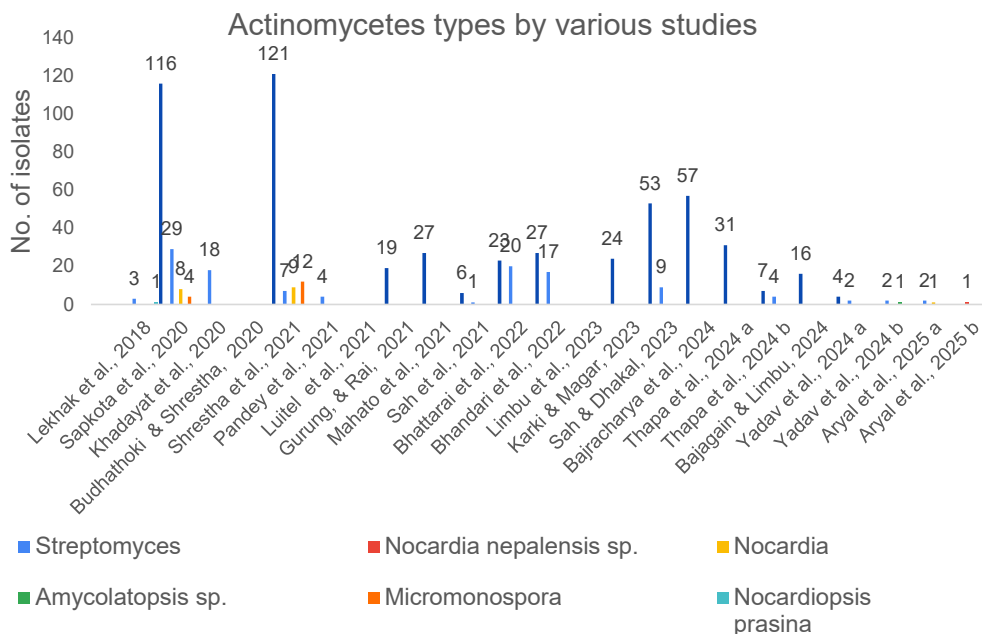


Chart 3

Characteristics of selected studies (2018-2025)



Many isolated actinomycetes strains showed inhibitory activity against various bacterial pathogens. The maximum zone of inhibition (ZOI) against *S. aureus* (33.33 mm and 34 mm), was observed in studies by Lekhak et al., 2018 and Thapa et al., 2024a. Similarly, the maximum ZOI against *Bacillus* spp. (26 mm and 24.67 mm) noted in the studies conducted by Limbu et al., 2023. The highest ZOI against *E. coli* was (26 mm and 34 mm, respectively) was reported in studies by Lekhak et al., 2018 and Thapa et al., 2024a. The maximum ZOI against *Salmonella* Typhi was 28mm and 30mm in studies by Yadav et al., 2024a and Thapa et al., 2024b respectively. The maximum ZOI against *Shigella sonnei* was 30 mm in studies by Bhattarai et al., 2022, Bhandari et al., 2022, Thapa et al., 2024b, Yadav et al., 2024a respectively. The highest ZOI against *K. Pneumoniae* was 18 mm in research studies by Sapkota et al., 2020. The highest ZOI against *P. aeruginosa* was 16 mm in research studies by Limbu et al., 2023. The highest ZOI against *A. baumannii* was 31.33 mm in research study by Lekhak et al., 2018 as shown in chart 4.

The research by Lekhak et al., 2018 showed significant activity against *Candida albicans* (ZOI 31.33mm). Similarly, the study by Yadav et al., 2024b had notable antifungal activity against *Aspergillus niger* (ZOI 18mm) and *Saccharomyces cerevisiae* (31 mm) as shown in chart 5. Research by Yadav et al., 2024a and Yadav et al., 2024b

revealed notable cytotoxic effects against HeLa and MCF-7 cell lines as shown in chart 6 (Aryal, et al., 2025a; Bhattarai et al., 2022; Budhathoki & Shrestha, 2020; Gurung & Rai, 2021; Lekhak et al., 2018; Mahato et al., 2021; Sapkota et al., 2020; Yadav, Bhattarai, et al., 2024; Yadav et al., 2024b). Activity against drug-resistant pathogens including MRSA and ESBL-producing *E. coli* was demonstrated in various studies, (Bhandari et al., 2022; Bhattarai et al., 2022; Budhathoki & Shrestha, 2020; Khadayat et al., 2020; Lekhak et al., 2018; Yadav, Bhattarai, et al., 2024). Some isolates demonstrated broad-spectrum efficacy against all kinds of bacteria (Bhattarai et al., 2022; Lekhak et al., 2018; Sapkota et al., 2020; Yadav, Bhattarai, et al., 2024). Other studies found higher sensitivity towards Gram-positive bacteria, particularly *S. aureus* (Gurung & Rai, 2021). In some studies the crude extracts proved more effective against Gram-negative bacteria (Shrestha et al., 2021).

Chart 4

Zone of inhibition (ZOI mm) of various Actinomycetes EA extracts against pathogenic bacteria

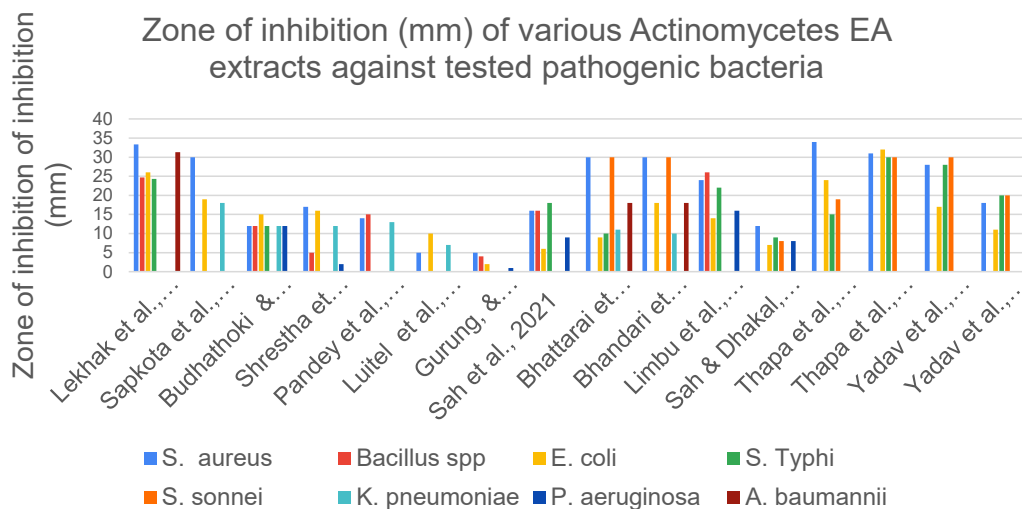
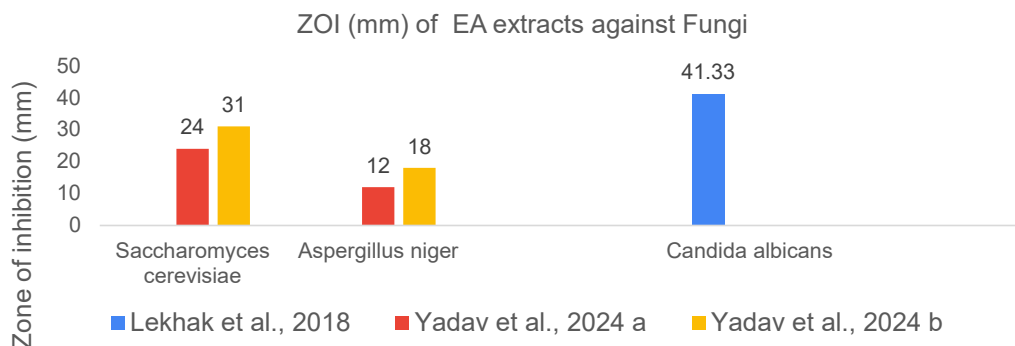
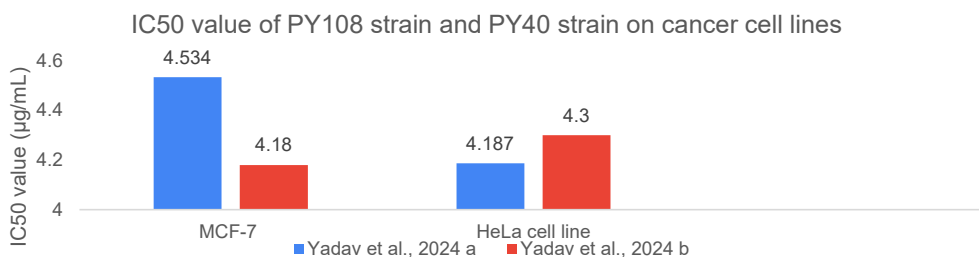


Chart 5

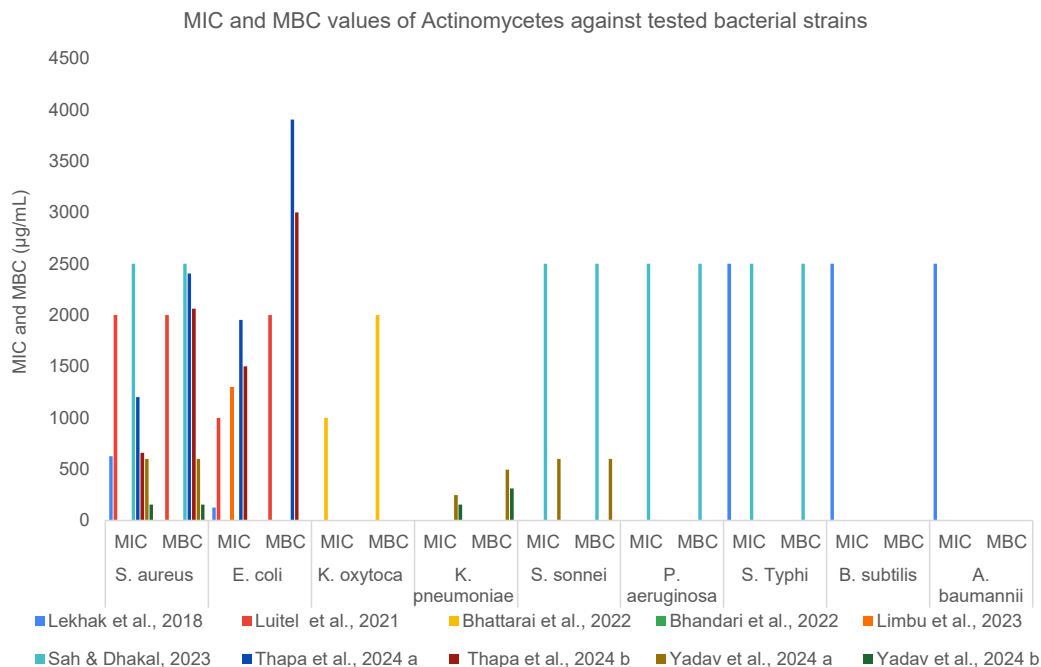
Zone of inhibition (ZOI mm) of EA extracts against Fungi

**Chart 6**

IC50 value of PY108 strain and PY40 strain on cancer cell lines



Water source isolates sometimes showed better antimicrobial properties than soil isolates (Shrestha et al., 2021). Isolates from higher altitudes and moist conditions were often associated with higher yield or potency (Bhandari et al., 2022; Bhattarai et al., 2022; Thapa, et al., 2024b). MIC and MBC values were determined for potent extracts, indicating the concentration required to inhibit or kill pathogens as shown in chart 7 (Bhandari et al., 2022; Bhattarai et al., 2022; Budhathoki & Shrestha, 2020; Lekhak et al., 2018, 2018; Thapa, et al., 2024b; Yadav, Bhattarai, et al., 2024; Yadav et al., 2024b).

Chart 7*MIC and MBC values of Actinomycetes against bacteria*

Various bioactive metabolites and chemical classes were identified in crude extracts of Nepalese actinomycetes using advanced techniques like LC-MS/MS and GC-MS (Aryal, et al., 2025a; Bhandari et al., 2022; Bhattarai et al., 2022; Thapa, et al., 2024a; Thapa, et al., 2024b; Yadav, Bhattarai, et al., 2024; Yadav et al., 2024b). The common metabolites identified in crude extracts across several studies were diketopiperazines, which have both antimicrobial and antitumor activities (Bhandari et al., 2022; Bhattarai et al., 2022; Thapa, et al., 2024a; Yadav, Bhattarai, et al., 2024; Yadav et al., 2024b). The polypeptide antibiotics such as actinomycin D and X2 recognized as chemotherapeutic agents have also been reported from Nepal (Bhattarai et al., 2022; Yadav et al., 2024b).

Other identified compounds include bacterial alkaloids, anthramycin type antibiotics such as abbeymycin, lipase inhibitors, cytotoxic metabolites, antifungal and antitumor antibiotics (including reductionmycin, streptimidone, deoxybomycin), alaremycin, fumaramidmycin, anisomyacin, mayamycin, nocardamine, streptazone D, nocardimicin B, melleolides, spathullin B, nannoquinone B, aureolic acid derivatives (like chromomycin A), lipopeptides (such as surfactin), and γ -sitosterol as shown in table 2 (Aryal, et al., 2025a; Bhattarai et al., 2022; Yadav, Bhattarai, et al., 2024; Yadav et al., 2024b). Various enzymes, including amylase, cellulase, gelatinase, lecithinase, and

urease, have been documented in Actinomycetes isolated from Nepal (Bajracharya et al., 2024; Pandey et al., 2021).

Table 2

Compounds identified in various studies

SN	References	Compound Identification	Method of Compound Identification
1	Bhattarai et al., 2022	54 secondary metabolites include diketopiperazines, actinomycin D, actinomycin X2, bacterial alkaloids (e.g., bohemamine), anthramycin antibiotics (e.g., abbeymycin), lipase inhibitors (e.g., ebelactone B), and antifungal/antitumor antibiotics (e.g., reductionycin)	GNPS, LC-HRMS
2	Bhandari et al., 2022	24 different secondary metabolites such as diketopiperazines and aminocoumarin antibiotics	GNPS, LC-HRMS
3	Thapa et al., 2024 a	22 metabolites such as epopromycin A, gilvocarcin HE	LC-HRMS
4	Thapa et al., 2024 b	37 metabolites such as diketopiperazines, surfactins, novel cyclopeptides	LC-MS/MS and GNPS
5	Yadav et al., 2024 a	27 specialized metabolites such as diketopiperazines, aureolic acid derivatives, lipopeptides, 34 metabolites including actinomycin D, γ -sitosterol.	LC-HRMS/MS, GC-MS
6	Yadav et al., 2024 b	Diketopiperazines and anticancer compound hexahydropyrrolo[1,2-a]pyrazine-1,4-dione	GC-MS
7	Aryal et al., 2025 a	Mayamycin, Nocardamine, Streptazone D, Nocardimicin B, 4-O-methylmelleolide, Spathullin B, Nannozinone B	HPLC, UPLC-MS/MS

Discussion

Actinomycetes yield a variety of secondary metabolites showing diverse biological activity and are known for their potential in the production of antibiotics (Lekhak et al., 2018; Sapkota et al., 2020). Modern treatment depends mainly on antibiotics for treating

bacterial diseases, but treating such diseases is made more difficult by the emergence of antibiotic resistance in pathogenic bacteria (Aryal et al., 2025b). Thus, in order to fight antibiotic resistant bacteria, new antibiotics/antimicrobials must be introduced. Antibiotic-resistant bacteria are becoming a growing problem worldwide, so it is critical to investigate alternative treatment options. The treatment of common infectious diseases is seriously threatened by new resistance mechanisms that are developing and spreading throughout the world, increasing the number of fatalities and disabilities. Therefore, investigating a new antibiotic is crucial to tackling the problems caused by infections.

Different studies conducted previously in Nepal confirm that soils and water possess diverse Actinomycetes strains that have ability to produce different bioactive compounds which have antimicrobial activity against various pathogens (Aryal, et al., 2025a; Bhandari et al., 2022; Bhattarai et al., 2022; Lekhak et al., 2018; Limbu et al., 2023; Mahato et al., 2021; Sapkota et al., 2020; Shrestha et al., 2021; Thapa, et al., 2024b; Yadav, Bhattarai, et al., 2024). The Actinomycetes from Nepal may represent an important resource for the discovery of novel antimicrobial agents, new antibiotics, and anticancer agents in light of the growing issue of drug resistance (Bhandari et al., 2022; Bhattarai et al., 2022; Khadayat et al., 2020; Yadav, Bhattarai, et al., 2024; Yadav et al., 2024b).

The unexplored habitats in Nepal including diverse niches at high altitudes are the most potential areas for the discovery of novel strains and potent bioactive compounds (Aryal, et al., 2025a; Bajracharya et al., 2024; Bhandari et al., 2022; Bhattarai et al., 2022; Luitel et al., 2021; Mahato et al., 2021; Sapkota et al., 2020; Thapa, et al., 2024b; Yadav, Bhattarai, et al., 2024). The initial characterization of actinomycetes can be established through morphological and biochemical traits, as well as 16S rRNA gene sequence analysis. However, the sequencing of the 16S rRNA gene alone is insufficient for species-level characterization of many Actinomycetes including *Streptomyces*. Additional techniques such as fatty acid methyl ester (FAME) analysis, metabolite profiling, and DNA-DNA hybridization methods are employed to identify Actinomycetes and *Streptomyces* at the species level (Khadayat et al., 2020; Sah & Dhakal, 2023).

Advanced analytical techniques are applied to continue the characterization of metabolites produced by these strains and to deduce potential bioactivities. These included LC-MS/MS and GC-MS in parallel with computational tools such as GNPS and SIRIUS. For elucidating of the complete structure of bioactive compounds, NMR is the most important tool. However, it lacks in these works (Aryal, et al., 2025a; Bhandari et al., 2022; Bhattarai et al., 2022; Thapa, et al., 2024a; Thapa, et al., 2024b; Yadav, Bhattarai, et al., 2024; Yadav et al., 2024b).

The identified metabolites diketopiperazines, polypeptide antibiotics, and others have already been reported with a vast array of biological activities, consequently

explaining the observed antimicrobial and cytotoxic effects of the crude extracts (Bhandari et al., 2022; Bhattarai et al., 2022; Thapa, et al., 2024a; Yadav, Bhattarai, et al., 2024; Yadav et al., 2024b). Further research is needed for isolation and characterization of individual compounds with respect to their bioactivity (Lekhak et al., 2018; Mahato et al., 2021; Yadav, Bhattarai, et al., 2024; Yadav et al., 2024b).

Conclusion

Various studies on soil Actinomycetes conducted in Nepal have identified that novel strains of Actinomycetes such as *Nocardiopsis prasina*, *Amycolatopsis* sp, *Nocardia nepalensis* sp. nov., and various strains of *Streptomyces* such as *Streptomyces violarius*, *S. krainskii*, *S. tsusimaensis*, *Streptomyces hundingensis*, *Streptomyces* sp. Ed-065 etc. was isolated and identified. These Actinomycetes isolates demonstrated notable antimicrobial activity against pathogens, multi drug resistant pathogens, fungal pathogens as well as notable cytotoxic effect on cancer cell lines. These studies suggest that Nepalese soil may be a source of novel bioactive compounds or antimicrobial compounds that could combat antimicrobial resistance. So, therefore ongoing search and characterization of actinomycetes from unexposed and less-studied environments in Nepal are essential. Such efforts are recommended as a part of national policy to discover noble bioactive and antimicrobial agents. Such research could contribute to finding cost-effective therapeutic solutions and addressing antimicrobial resistance.

Conflict of interest

In this study there is no conflict of interest.

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