Research Article

Unveiling Antibacterial Potential of Actinomycetes against Drug-Resistant Pathogens

Nataraja. B.T[1], Ramalingappa B[2] , Sowmya K.L[3]

[1]Department of Microbiology, Maharani's Science College for Women, Mysore.
[2]Davangere University, Department of Studies in Microbiology, Davangere-577007, Karnataka, India.
[3]Davangere University, Department of Studies in Microbiology, Davangere-577007, Karnataka, India

(Received: 21-10-2024 Revised: 18-12-2024 Accepted: 02-01-2025)

Corresponding Author: ***Ramalingappa B***. Email: ramalingappa.88@gmail.com

ABSTRACT

Introduction and aim: Actinomycetes are widely recognized for their remarkable ability to produce a wide array of secondary metabolites, including antibiotics, antitumor agents, and other biologically active compounds. The document emphasizes the ubiquity of these filamentous microorganisms in soil and highlights the screening of actinomycetes for antimicrobial activity, involving primary and secondary screening methods. It details the steps involved in primary screening, such as the cross-streak assay method, and the subsequent secondary screening for antibiotic production. The selection of actinomycete strains for mass cultivation and the extraction of antimicrobial metabolites is also outlined. The aim of the study is to shed light on the multifaceted role of actinomycetes, encompassing their antimicrobial properties, through a comprehensive exploration of the isolation, purification, and characterization of actinomycetes, the document aims to provide valuable insights into their potential as sources of bioactive compounds and their significance in addressing challenges such as antimicrobial resistance and the need for new therapeutic agents.

Materials and Methods: The study addresses the antagonistic activity of actinomycetes against various pathogenic bacteria, emphasizing their potential in combating drug-resistant pathogens and also provides a detailed account of the methods used to assess antagonistic activity, including agar diffusion methods.

Results: The results highlights the significant findings of the study, including the isolation, purification, and characterization of actinomycetes from soil samples, emphasizing their antimicrobial activity against pathogenic strains.

Conclusion: The study concludes that actinomycetes exhibit significant biodiversity and possess strong antibacterial properties, particularly against drug-resistant bacteria. The isolation and characterization of these microorganisms highlight their potential as sources of new antibacterial compounds and their role in addressing antibacterial resistance.

Keywords: Actinomycetes, Antimicrobial activity, Secondary metabolites, Antibiotics, Agar diffusion method.

1. INTRODUCTION

Actinomycetes, a diverse group of gram-positive bacteria, have long been recognized for their remarkable ability to produce a wide array of secondary metabolites, including antibiotics, antitumor agents, and other biologically active compounds [1]. These filamentous microorganisms are ubiquitous in the soil and play a crucial role in the recycling of organic matter, making them an essential component of terrestrial ecosystems. One of the most significant aspects of actinomycetes is their potential as plant growth-promoting microbes. Certain strains of actinomycetes can directly stimulate plant growth through the production of phytohormones, such as auxins and cytokines, as well as by enhancing nutrient availability [2]. Additionally, actinomycetes have been found to exhibit antifungal and antibacterial properties,

making them effective biocontrol agents against plant pathogens [3]. The versatility of actinomycetes extends beyond their agricultural applications, as they have also been widely explored for their potential in the medical field. Actinobacteria, particularly the genus Streptomyces, are known to be prolific producers of bioactive compounds with antimicrobial, antitumor, and anti-inflammatory properties. As a result, actinomycetes have been the focus of extensive research, with the aim of discovering new therapeutic agents to address the growing challenges posed by drug-resistant pathogens and the increasing incidence of cancer [4]. The ability of actinomycetes to produce an extensive variety of secondary metabolites, many of which have antibacterial or antifungal properties, and the formation of mycelia during sporulation are two of their unique biological traits. Some Streptomyces both sporulate cells and produce antibiotics. In general, Streptomyces sp. grows best in media that contain carbon and nitrogen sources such as nitrate, chitin, starch, glycerol, arginine, asparagine, and casein [5]. Streptomyces are especially prevalent and can produce a variety of antibiotics and other physiologically active types of secondary metabolites. Many harmful fungus and bacteria have developed resistance to widely used antibiotics. Research on this antibacterial and antifungal resistance is currently urgently needed, as new medications are required to combat these infections. The ability of actinomycetes filamentous soil bacteria to generate a large variety of secondary metabolites with varied chemical structures and biological activity, including antibiotics, has led to their widespread recognition as significant microorganisms [6]. The 1970s saw a boom in the discovery of new antibiotics, which declined in the late 1980s and early 1990s primarily because compounds ran out but rather because of a reduction in screening efforts. This study looked into isolating soil actinomycetes with antibacterial (for Gram negative and Gram-positive bacteria) or antifungal (for mold and yeast) properties from understudied regions.

2. MATERIALS AND METHODS

2.1 Collection of soil samples: Samples of soil are being collected from many Bellary mines areas in the Chitradurga district. To isolate actinomycetes, the obtained soil samples were placed in sterile plastic bags.

2.2 Pre-treatment of soil samples: Pre-treatment was applied to the soil sample to lower the percentage of microorganisms other than actinomycetes. The samples of dirt were dried for around 5-10 minutes at 50–600C [7] [8].

2.3 Isolation and Identification of actinomycetes: Benedict's agar medium, Starch Casein nitrate agar medium, Actinomycetes isolation agar medium was used for the isolation of actinomycetes. 100ml of sterile distilled water was used to suspend 1g of each soil sample, homogenize it by vortex mixing, allow the compositions settle, and then develop serial tenfold dilutions up to 10-4 using sterile distilled water [9]. On actinomycetes isolation agar plates, isolation was accomplished via spreading. For ten days, the plates were incubated at 25°C. Once the colonies reached a powdery development stage, they were observed. To obtain an axenic culture, colonies were chosen and re-spread on an actinomycetes isolation agar medium based on morphological variations and colour. The spore stocks were taken from the culture grown on actinomycetes isolation media and stored in the refrigerator in anticipating of further antagonistic studies [10] [11]. Macroscopic and microscopic characteristics of actinomycetes was observed under microscope. Identification of actinomycetes was carried out by biochemical tests [12].

2.4 Actinomycetes screening for antimicrobial activity: Primary and secondary screening are the two main stages in the process of evaluating actinomycetes for antibacterial activity.

2.4.1 Primary Screening of actinomycetes: The antagonistic activity was assessed using the cross-streak assay technique. On the modified nutrient agar plates, a single, 4-6 mm-diameter stripe of the isolated actinomycetes was cultivated for five to seven days at room temperature (28 ± 20°C). A variety of bacteria were streaked perpendicular to the actinomycetes

initial streak and grown for a full day at 28±20°C after a ribbon-like growth was noticed. The growth of every organism was found to be impeded. In order to assess the normal growth of bacteria and fungi, a control plate devoid of actinomycetes was also maintained. The antagonistic activity of each actinomycetes isolated from the different soil samples was examined [13].

2.4.2 Secondary screening for antibiotic production: Five actinomycetes strains RLNBT1, RLNBT2, RLNBT3, RLNBT4, and RLNBT5 were selected for mass cultivation and the extraction of the required quantity of antimicrobial metabolites because of their more promising antagonistic activity. A loopful of selected actinomycete strains was inoculated into a 250 ml conical flask that was filled with 100 ml of glucose soybean medium. After that, the flask was continuously shaken and kept at 28°C for 72 hours. Then, in a 500 ml conical flask, 20 ml of each broth culture were transformed into 200 ml of soybean mean broth, which was shaken constantly for seven days [14].

2.5 Antibacterial metabolites isolation: Using the solvent extraction procedure, the antibacterial component was extracted from the filtrate and recovered in the manner outlined. For full extraction, ethyl acetate was added to the filtrate in a 1:1 ratio and agitated vigorously for one hour. The antibiotic-containing ethyl acetate phase was isolated from the aqueous phase. It was dried in a water bath at 800-900 degrees Celsius, and the resulting residue was weighed. The resulting chemical was utilized for bio assignment, determining minimal inhibitory concentration, and assessing antibacterial efficacy [15].

2.6 Determination of antimicrobial activity: *Staphylococcus aureus, Pseudomonas sp., Klebsiella, E. coli, Bacillus sp., and Streptococococcus species* were used to detect the antibacterial activity using the agar well method. The partially purified extract that resulted from the evaporation of the ethyl acetate extract was dissolved in phosphate buffer. The sample was subsequently placed into wells on swabbed Muller Hinton agar plates that contained test organisms. The plates were examined and the diameter of the zones of total inhibition was evaluated following 18 to 24 hours of incubation at 37°C [16].

2.7 Effect of Different Nitrogen, Carbon Sources: Other nitrogen sources, such as peptone and potassium nitrate and carbon sources such as Fructose, Sucrose and mannitol were added to the specified culture broth in place of the original nitrogen source. The inoculated medium was incubated at room temperature for five days and the culture filtrates were then analyzed [17].

2.8 Impact of pH on Actinomycetes Production of Antibiotics: In order to examine the impact of pH on antibiotic production, the pH values of the chosen medium were altered using hydrochloric acid prior to sterilization, and they ranged from 5 to 9. It was assessed after five days of incubation at room temperature [17].

3. RESULTS

Benedict's agar medium, Starch Casein nitrate agar medium, Actinomycetes isolation agar medium was used for isolation of actinomycetes from various soil samples. During isolation among different media, Starch casein nitrate agar medium showed confluent growth of powdery colonies after 6 to 7 days of incubation period. The morphological characteristics of actinomycetes was observed (Table.1).

Table.1. Cultural characteristics of actinomycetes strains on different isolation media.

Strain	Starch Casein agar		Benedict's agar		Actinomycetes isolation agar	
	Growth	Mycelium colour	Growth	Mycelium colour	Growtrh	Mycelium Colour
RLNBT1	+++	White	++	Grey	++	Grey
RLNBT2	+++	White	++	Grey	++	White
RLNBT3	++	White	++	Greyish white	+++	White
RLNBT4	++	Grey	+	White	++	Grey
RLNBT5	+++	Grey	++	Grey	++	White

Only five of the thirty-two isolates shown action against the test organisms. The impact of the incubation duration on the biosynthesis of antibiotics by a selection of five actinomycetes was shown in Table 2. Beginning on the second

day of incubation, antibacterial activity was seen, attaining its peak on the sixth day of incubation.

Table: 2. Effect of incubation period on antibiotic biosynthesis by actinomycetes strains.

Strain	Incubation time	Test organisms			
		E.coli	*Pseudomonas sp*	*Bacillus sp*	*S.aureus*
		Zone of Inhibition in mm			
RLNBT1	2	-	-	-	-
	3	5	-	-	9
	4	11	1	-	15
	5	19	3	-	23
	6	21	5	5	25
	7	25	6	9	31
RLNBT2	2	-	-	-	-
	3	6	-	-	5
	4	10	-	-	6
	5	18	-	6	9
	6	20	-	9	12
	7	23	-	11	19
RLNBT3	2	-	-	-	-
	3	7	-	5	5
	4	9	-	7	9
	5	12	3	9	13
	6	15	4	11	17
	7	19	5	15	23
RLNBT4	2	-	-	-	-
	3	-	-	4	5
	4	3	-	8	10
	5	6	-	12	15
	6	11	3	19	21
	7	15	5	21	29
RLNBT5	2	-	-	-	-
	3	3	-	6	7
	4	6	-	12	11
	5	9	5	18	15
	6	12	9	25	19
	7	15	15	31	21

Five actinomycetes strains showed highest activity against *E.coli, Bacillus sp, S.aureus* but less activity shown by *Pseudomonas sp.* The synthesis of antibiotics depends on the medium's pH. When comparing pH5, pH7, and pH9, Figure 1, 2, and 3 demonstrated that the isolated actinomycetes strains produced antibiotics after 72 hours of fermenting broth. pH 5, pH 7 and pH 9 showed maximum antibiotic effect by *Bacillus sp.* followed by *Staphylococcus aureus, E.coli* and less activity by *Pseudomonas sp.* pH 9 showed maximum activity compared to pH5 and pH7.

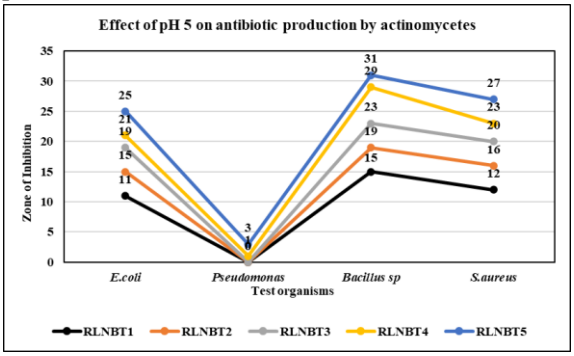

Figure: 1. Effect of pH 5 on antibiotic production by actinomycetes strains.

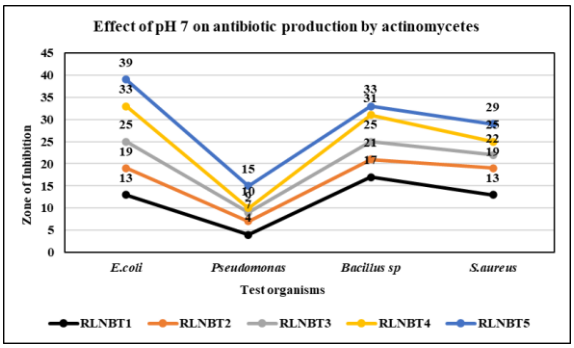

Figure: 2. Effect of pH 7 on antibiotic production by actinomycetes strains.

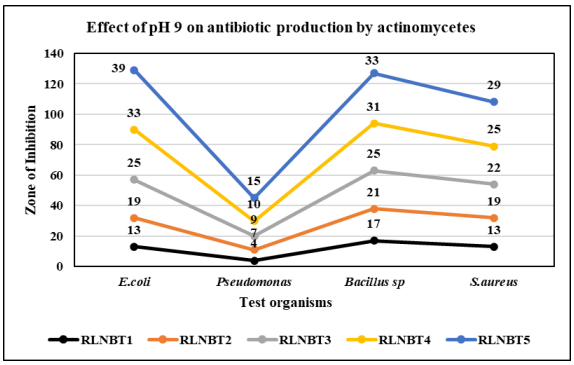

Figure: 3. Effect of pH 9 on antibiotic production by actinomycetes strains.

As seen in Figure 4 to Figure 8, medium supplied with sucrose and fructose showed higher levels of antibacterial biosynthesis, while medium supplemented with mannitol showed lower levels. The actinomycetes strains RLNBT1, RLNBT2, RLNBT3, RLNBT4, and RLNBT5 had the strongest antibacterial activity against *E. coli, Bacillus species, and S. aureus, whereas Pseudomonas species* exhibited the least effective antibacterial activity.

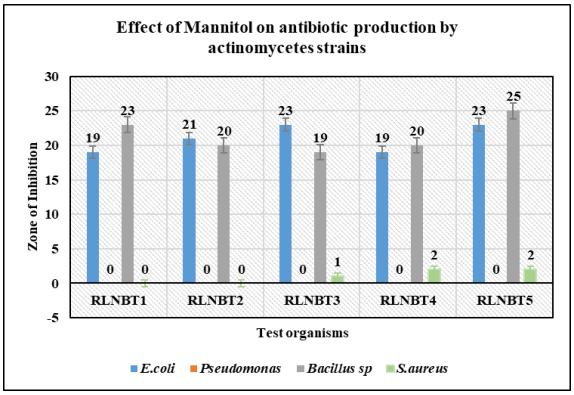

Figure: 4. Effect of Mannitol on antibiotic production by actinomycetes strains.

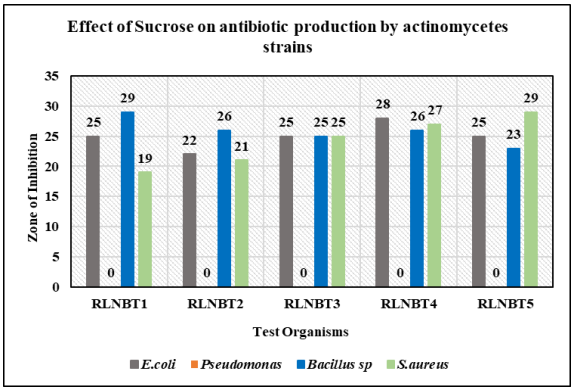

Figure: 5. Effect of Sucrose on antibiotic production by actinomycetes strains.

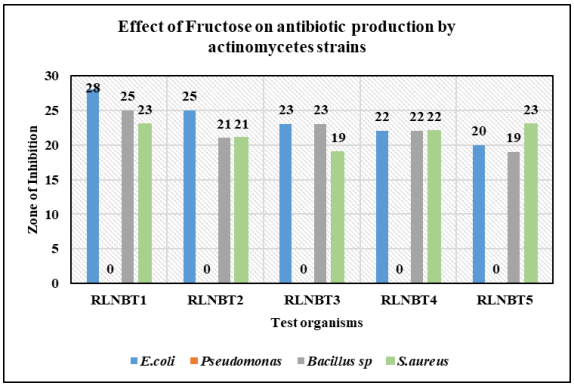

Figure: 6. Effect of Fructose on antibiotic production by actinomycetes strains.

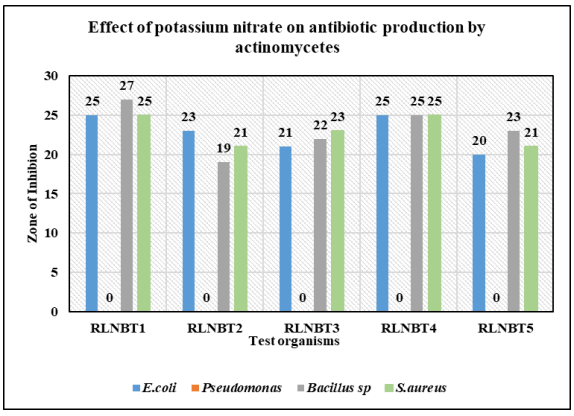

Figure: 7. Effect of Potassium nitrate on antibiotic production by actinomycetes strains.

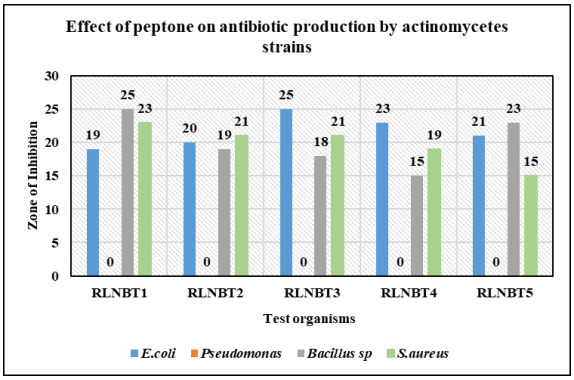

Figure: 8. Effect of Peptone on antibiotic production by actinomycetes strains.

4. DISCUSSION

Selvamohan *et al.,* [17] investigates the isolation of actinomycetes from soil samples to analyse their antagonistic activity against various pathogens and explained the collection and pre-treatment of soil samples, and pre-treated to reduce other microorganisms, followed by suspension in sterile distilled water and vortex mixing. Serial tenfold dilutions were prepared, and the samples were plated on actinomycetes isolation agar, which promoted rapid growth of powdery colonies. Antagonistic activity was assessed using cross-streak assays on modified nutrient agar plates, followed by agar diffusion methods to evaluate the effectiveness of the isolated actinomycetes against various pathogenic bacteria. Sowmya and Ramalingappa [9], focused on isolating actinomycetes from different soil samples in Davangere City, resulting in the collection of 65 isolates. The highest number of isolates was obtained from Starch Casein Nitrate Agar Medium (SCNAM), with 20 strains, while varying colony morphologies, including white, buff, and brownish colonies, were observed. The research demonstrated the effectiveness of the serial dilution method for isolating actinomycetes, reinforcing their significance in producing antibiotics and other bioactive compounds. Chaudhar *et al.,* [18] discusses the isolation, purification, and characterization of actinomycetes from soil samples in Sheopur, Madhya Pradesh, India, focusing on their antimicrobial activity against 12 pathogenic strains. Morphological characterization of actinomycete isolates was performed by inoculating them on seven different ISP media (ISP1-ISP7) and incubating for 5 days at 30°C. The colonies were then observed under a high-power magnifying lens, noting characteristics such as colour, aerial and substrate mycelium, branching patterns, and overall colony morphology. This assessment provided insights into the diversity and classification of the actinomycete isolates based on their physical appearance. A total of 31 actinomycete isolates were identified, with several demonstrating significant antibacterial properties, particularly

against drug-resistant bacteria and their study highlights the potential of these isolates as sources of new antibacterial compounds, emphasizing the biodiversity of the Sheopur region. Actinomycetes were isolated by suspending dried soil in sterile water, serially diluting, and plating on actinomycete isolation agar, followed by incubation. Antibacterial activity was assessed using the agar well diffusion method, and minimum inhibitory concentration (MIC) was determined through serial dilution in microtiter plates. Sowmya and Ramalingappa [12], discussed the biodiversity of actinomycetes in various environments and their potential applications and explained about the isolation and identification of actinomycetes from different soil samples, as well as the characterization of these microorganisms through various tests such as gram staining, motility, and biochemical tests and also delves into the production of enzymes by actinomycetes and their potential industrial applications. It covers the development of an environmentally friendly bio fertilizer using actinomycetes and provides valuable insights into the diversity, characteristics, and potential uses of actinomycetes.

5. CONCLUSION

The conclusion of the manuscript emphasizes the significant biodiversity and strong antimicrobial properties of actinomycetes, particularly in combating drug-resistant bacteria. It underscores the potential of actinomycetes as sources of new antibacterial compounds and their role in addressing challenges such as antimicrobial resistance. The document also highlights the multifaceted role of actinomycetes, encompassing their antimicrobial properties and their potential as sources of bioactive compounds. Overall, the conclusion underscores the importance of actinomycetes in the field of microbiology and their potential applications in agriculture and the medical field, particularly in the production of antibiotics and other bioactive compounds.

CONFLICT OF INTEREST

The authors declare that there is no Conflict of Interest.

ACKNOWLEDGMENT

We would like to thank Davangere University, Department of Studies in Microbiology for providing the research space.

FUNDING

There is no funding.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

REFERENCES

1. Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G.F., Chater, K.F. Genomics of actinobacteria: Tracing the evolutionary history of an ancient phylum. *Microbiology and Molecular biology Reviews.* 2007; 71:495-548.
2. Bhatti, A.A., Haq, S., Bhat, R.A. Actinomycetes benefaction role in soil and plant health. *Microbial Pathogenesis.* 2017; 111:458-467.
3. Pandey, A., Ali, I., Butola, K.S. Chatterji, T., Singh, V. Isolation and characterization of actinomycetes from soil and evaluation of antibacterial activities of actinomycetes against pathogens. *International Journal of Applied Biology and Pharmaceutical Technology.* 2011; 2(4):18-32.
4. Amin, D. H., Abdallah, N. A., Abolmaaty, A., Tolba, S., Wellington, E. M.

Microbiological and molecular insights on rare Actinobacteria harboring bioactive prospective. *Bulletin of the National Research Centre.* 2020; 44(1):1-12.

5. Balachandran, C., Duraipandiyan, V., Emi, N., Ignacimuthu, S. Antimicrobial and cytotoxic properties of *Streptomyces sp.* (ERINLG-51) isolated from Southern Western Ghats. *South Indian Journal of Biological Science.* 2015; 1(714):1-5.
6. Singh, L.S., Sharma, H., Talukdar, N.C. Production of potent antimicrobial agent by actinomycete *Streptomyces sannanensis* strain SU118 isolated from phoomdi in Loktak lake of Manipur, India. *BMC Microbiology*. 2014; 14: 278.
7. Hong, K., Gao, A,. Xie, Q., Gao, H., Zhuang, L., Lin, H., Yu, H., Li, J., Yao, X., Goodfellow, M., Ruan, J. Actinomycetes for Marine Drug Discovery Isolated from Mangrove Soils and Plants in China. *Marine Drugs*. 2009; 7:24-44.
8. Matsukawa, M., Nakagawa, Y., Limura, Y,. Hayakawa, M. A New Enrichment Method for the Selective Isolation of Streptomycetes from the Root Surfaces of Herbaceous Plants. *Actinomycetologica*. 2007; 21: 66-69.
9. Sowmya, K.L., Ramalingappa. Isolation of Actinomycetes from Different Soil Samples of Davangere City. *International Journal of Science and Research.* 2020; 11(2):1068-1074.
10. Rahman, M.A., Islam, M.Z., Islam, M.A. Antibacterial activities of Actinomycetes isolates collected from soils of Rajshahi, Bangladesh. *Biotechnology Research International.* 2011; 857925.
11. Pine, L. Watson, S.J. Evaluation of an isolation and maintenance medium for Actinomycetes species and related organisms. *Journal of Laboratory and Clinical Medicine.* 1959; 54:107-114.
12. Sowmya, K.L., Ramalingappa. Biochemical Characterization of Actinomycetes Isolated from Different Soil Samples. *International journal of research in academic world.* 2022; 1(15):11-17.
13. Rai, M., Bhattrai, N., Dhungel, N and Mandal, P.K. Isolation of antibiotic producing actinomycetes from soil of Kathmandu valley and assessment of their antimicrobial activities. *International Journal of Microbiology and Allied Sciences.* 2016; 2(4): 22-26.
14. Yadav, U. T., Shrestha, K. B., Tiwari, G. S., Sahukal, and Agrawal, V.P. Streptomycin-like antibiotic from *Streptomyces spp* isolated from Mount Everest base camp. *Nepal Journal of Science and Technology.* 2008; 9: 73-75.
15. Gurung, T.D., Sherpa, C., Agrawal, V.P., Lekhak, B. Isolation and characterization of antibacterial actinomycetes from soil samples of Kalapatthar, Mount Everest region. *Nepal Journal of Science and Technology.* 2009; 10:173-182.
16. Ganesan, R. H. A., David, A. D., Reegan. Isolation and molecular characterization of actinomycetes with antimicrobial and mosquito larvicidal properties. Beni-Seuf University *Journal of Basic and Applied Sciences.* 2017; 6(2): 209-217.
17. Selvamohan, T., Parameswaran, N.K., Abisha Felcy, S. Isolation of actinomycetes from different soils for analysing the antagonistic activity against pathogens. *International Journal of Advanced Research in Biological Sciences.* 2016; 3(8): 124-131.
18. Hotam, S., Chaudhary, Jayprakash Yadav, Anju, R., Shrivastava, Smriti Singh, Anil, K., Singh, Natrajan Gopalan. Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). *Journal of advanced pharmaceutical technology and research.* 2013; 4(2): 118-123.