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Screening of Actinomycetes From Soil for Antibacterial Activity

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Abstract

Actinomycetes are Gram positive, free living saprophytes which are distributed in soil as one of the major populations and are primary source of antibiotics. This study was carried out with a quest to isolate actinomycetes from soil samples of different places and assess their antibacterial activity. Isolation of actinomycetes was carried out by serial dilution of soil sample followed by spread plate method. The antimicrobial extract was extracted using ethyl acetate. Assessment of antimicrobial activity was performed by using Agar cup plate assay against test organisms (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi, Bacillus subtilis, Staphylococcus aureus*). Antibacterial activity was tested against Methicillin Sensitive *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* in the isolates having effective inhibitory activity against *Staphylococcus aureus*. From 15 soil samples of 12 different locations, 121 actinomycetes isolates were isolated. Among them, 58 (47.9%) isolates were inhibitory against at least 1 test organism in primary screening, of which 22 isolates effective against more than 1 test organism was chosen for secondary screening. Out of them, 8 were inhibitory against 2 test organisms while 14 were inhibitory against 3 test organisms. *Staphylococcus aureus* was found to be the most susceptible test organism with its susceptibility against 12 actinomycetes isolates. Among 12 isolates effective against *Staphylococcus aureus*, 10 were found to have an inhibitory effect against Methicillin Susceptible *Staphylococcus aureus* while 6 were found to have inhibitory effect against Methicillin Resistant *Staphylococcus aureus* strain. The findings of this study highlight the inhibitory potential of actinomycetes and the need forfurther investigation for obtaining novel antimicrobial agents from actinomycetes from various unexplored areas.

Keywords: Actinomycetes, Inhibitory activity, Isolates, Antimicrobial, Antibiotic

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Introduction

Year by year microbial diseases are increasing, which have become the biggest threat to public health. There are about 200 known diseases transmitted by microorganisms such as bacteria, fungi, viruses, etc. to humans [1]. Antibiotic refers to the chemical compound derived from microorganisms or living cells that inhibit and/or stop the growth of a microorganism. They are used in the treatment of external or internal infections. While some antibiotics are produced by a microorganism, most are now manufactured synthetically [2]. Bacteria, fungi, actinomycetes, algae, lichens and green plants produce antibiotics. Actinomycetes are one of the most important microorganisms that hold the prime position in the production of bioactive metabolites. They are responsible for the production of almost half of the discovered bioactive secondary metabolites notably antitumor agents, immunosuppressive agents, enzymes and especially antibiotics [3]. In industrial microbiology, actinomycetes make its position at the top as a potential source of antibiotics [4].

Actinomycetes are prokaryotic organisms with filamentous nature, branching pattern, and conidia formation, which are like those of fungi. For this reason, they are also known as Ray fungi. They are gram-positive, free-living, saprophytic bacteria [5, 6]. Actinomycetes populations are identified as one of the major groups of soil population, which may vary with soil type [7]. The number and types of actinomycetes present in soil would be greatly influenced by geographical location such as soil temperature, soil type, soil pH, organic matter content, cultivation, aeration and moisture content [8]. These are inhabitants of soil that also play important role in recycling of organic matter, production of novel pharmaceuticals, nutritional materials, cosmetics, enzyme inhibitors, immune-modifiers and vitamins [9, 10].

Numbers of antibiotics have now been isolated from cultures of actinomycetes, such as actinomycetin, micromonosporin, mycetin, and actinomyces lysozyme etc. have been only partially purified, whereas others, including actinomycin, proactinomycin, streptothricin, and streptomycin, have been isolated and crystallized These substances differ greatly chemical structure and their antimicrobial properties, toxicity to animals, and in vivo activity [11]. Compounds isolated from actinomycetes have numerous chemical structures such as macrolides, tetracyclines, aminoglycosides, glycopeptides and ansamicines which are used in

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antibacterial treatments whereas anthracyclines supplement anticancer chemotherapy. Toxic polyether-type antibiotics are used as anti-coccidal agents [12]. More than 70–80% of all known antibiotics have been isolated from actinomycetes, which are used in medicine and agriculture. The two major groups of soil actinomycetes that serve as important sources of antibiotics are *Streptomyces* and *Micromonospora*. *Streptomyces* spp. are the biggest producer of antibiotics that account for about 80% of the total antibiotic products. *Micromonospora* closely follows with less than one tenth as much as *Streptomyces* [1]. Members of *Streptomyces* are a rich source of bioactive compounds, notably antibiotics, enzymes, enzyme inhibitors and pharmacologically active agents [13]. Streptomyces are valuable resources for a novel products like antifungals, antivirals, antitumorals, anti-hypertensives, immunosuppressants and especially antibiotics [14].

The nature of the active agents or the antibiotics produced by actinomycetes depends upon the species; frequently upon the strain; the composition of the medium in which it is grown, and the conditions of cultivation. The antimicrobial properties of a given actinomycetes culture also depend upon the composition of the medium in which it is grown [11]. Also, it is essential to maintain the optimum temperature, pH and salinity to produce bioactive secondary metabolites. The absence of optimum conditions can lead to failure in production or no growth could also be observed [15].

However, with the time of the discovery of antibiotics, the emergence antibiotic resistance bacteria have been a major problem. Also, there is a rapid emergence of drug resistant strains of the pathogen than the rate of discovery of new drugs and antibiotics [16]. The development of resistance by the pathogens as well as the emergence of new pathogens has led to the necessity for the discovery of new antibiotics/antimicrobials for their infection. Hence screening of antimicrobial activity of actinomycetes and study of their antimicrobial action against pathogens is an important process for the discovery of an antibiotic.

In Nepal, various infections are a major public health problems. Antibiotics may be prescribed by the physician and other healthcare workers inappropriately or they may be purchased directly by consumers without prescription to the healthcare system. Many patients self-treat with antibiotics, including prior to hospital admission, which can contribute to increased resistance rate [17]. Drug resistance in microorganisms has been increasing and this has posed a serious threat for the mankind. Discovery of the novel antimicrobials that can control the growth of these microorganisms is of global importance. As, actinomycetes are the most important source of antibiotics this study is intended to find out the antimicrobial activity of actinomycetes and their potential to inhibit the microbial growth. This study will serve as baseline data for future studies of the antimicrobial potential of actinomycetes isolates and the discovery of novel metabolites from actinomycetes in Nepal.

Materials and Methods

Collection of Sample

15 soil samples from 12 different locations (Nuwakot, Lagankhel, Katunje, Chovar, Lubhu, Budanilkantha, Chapagaun, Godawari, Sundarijal, Sankhamul, Gokarna and Chandragiri) were collected for study (**Figure 1**). Laboratory processing of soil samples was carried out at Research Laboratory of Kathmandu Centre for Genomics & Research Laboratory (KCGRL) and Microbiology Laboratory of St. Xavier's College. Duplicate soil samples from a depth of 5-10 cm from the surface were collected in two separate sealed plastic containers [4, 14].

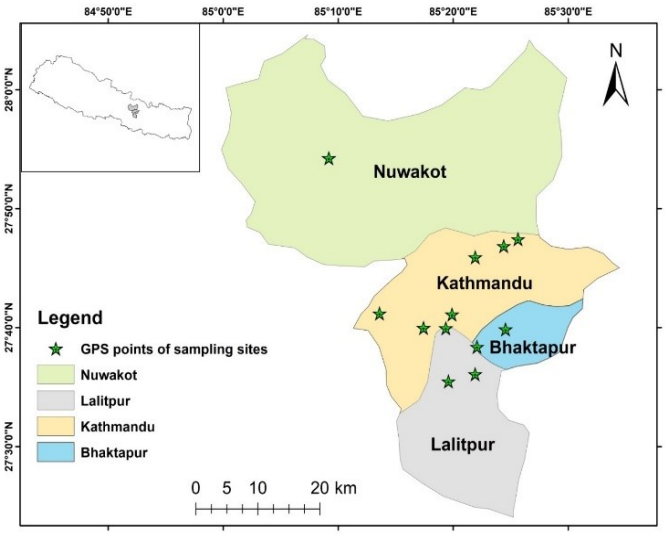

Figure 1. Map of sample collection sites

Soil treatment (before isolation)

Soil samples so collected were left to air dry at room temperature for (10-15) days, to reduce the number of contaminant bacteria (especially Gram-negative bacteria) in soil samples as previously reported [15, 18].

Isolation of Actinomycetes

The soil sample was serially diluted to 10^{-2}, using distilled water as diluents. The dilutions were vigorously shaken in a vortex shaker to liberate actinomycetes spores from the soil into the supernatant liquid. Aliquots of supernatant liquid from 10^{-1} and 10^{-2} dilutions of soil sample was plated on selective, solidified, Starch Casein Agar (SCA) incorporated with Amoxicillin 20 mg/l as reported previously [9] and Ketoconazole 30 mg/l [4] so

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as to eliminate contaminant gram positive soil dwelling bacteria and fungi respectively, using spread plate method. The inoculated SCA plates were left at room temperature for 5 minutes to allow the agar to absorb the liquid and were incubated at 28°C for 7 days [19].

Sub-culture of actinomycetes isolates

After 7 days of incubation at 28°C, colonies exhibiting a dry chalky appearance were selected. Starch Casein Agar (SCA) plate was divided into a number of sections and each of characteristic actinomycetes colonies was sub-cultured in sections of SCA plate. The plates were incubated at 28°C for 7 days. After colonies had developed, they were further sub-cultured in a test tube containing SCA slants, incubated at 28°C for 7 days and then stored in the refrigerator as the stock [19]. The isolates were sub-cultured again at an interval of 2-3 weeks to prevent the cultures from dying out.

Primary Screening of isolates

The duplicate perpendicular streak method was employed for the primary screening of inhibitory action of isolates against bacterial species. Actinomycetes isolates were streaked vertically down the center of the Nutrient agar plate and then incubated at 28°C until visible growth of actinomycetes appear as a confluent vertical line of the colony (usually 7 days). After incubation, each of the test organisms (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi, Bacillus subtilis, Staphylococcus aureus*), isolated from hospital admitted patients and confirmed with microscopic, cultural and biochemical characteristics [20], were streaked 1 cm apart and 2mm on the side of actinomycetes colony on nutrient agar, perpendicular to the colony and then incubated at 37°C for 24 hours. After incubation, plates were observed for any inhibition in the growth of test organisms [1, 6]. Inhibition in growth was noted as a lack of growth of test organisms on the streak line.

Selection of isolates for Fermentation:

Isolates inhibiting two or more test organisms (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi, Bacillus subtilis, Staphylococcus aureus*) in primary screening were selected for further processing i.e. fermentation for production of antibiotic fractions.

Fermentation

Among isolated actinomycetes, 22 isolates exhibiting the greatest action against test organisms in primary screening were selected based on comparative inhibition against one or more isolates and the ability to inhibit two or more test organisms. Yeast Extract- Malt Extract broth (ISP-2) was prepared in a conical flask. The colony of selected actinomycetes was cut from their pure culture and put into the broth. The conical flasks were placed into a rotary shaker incubator at 28°C for 8 days. [13, 21]

Extraction of antibiotic fraction:

After incubation, the broth was collected and centrifuged at 4000 rpm for 30 minutes. The supernatant was collected and an equal amount of ethyl acetate was added to it. The two phases were vigorously shaken for 1 hr. Then, the mixture was poured into a separating funnel and allowed to stand for 5 minutes during which aqueous and organic phases separated. The lower aqueous phase was discarded, while the upper organic phase was collected and heated in a water bath at 40°C to evaporate ethyl acetate [22]. The residue so left was weighed and dissolved in a small amount of phosphate buffer of pH 7 to solubilize crude antibiotic extract [13]. The mixture was transferred to an Eppendrof tube and stored at refrigerator temperature for further use.

Secondary Screening:

Agar well diffusion method was employed for secondary screening of inhibitory action against test organisms. Test organisms (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi, Bacillus subtilis, Staphylococcus aureus*) were inoculated into the nutrient broth and incubated at 37°C for 4 hrs. After incubation, the turbidity of the broth was compared with McFarland standard 0.5. Lawn/Carpet culture of test organisms was performed on Mueller Hinton Agar plates from McFarland adjusted broth cultures. Wells of 8 mm was bored in Mueller Hinton Agar plates using sterile agar borer and 40 µl of ethyl acetate extracted antibiotic fractions were added into wells. Plates were left at room temperature for 20-30 mins to diffuse antibiotic fractions, and then incubated at 37°C for 24 hrs. After 24 hrs, plates were examined and the diameter of the zone of inhibition around each well was measured [13].

Results

Soil harvest large number of antibacterial actinomycetes

From 15 soil samples, 121 isolates of actinomycetes were obtained. Among them, 58 isolates were found to have effective inhibitory activity against at least 1 test organism while 63 were ineffective against test organisms (**Table 1**). Twenty-two isolates produced observable inhibitory effects against two or more test organisms during primary screening (**Table 2**).

Among 58 isolates exhibiting inhibitory activity, 36 were active against one test organism and 22 against more than one test organism. Of those 22, 8 and 14 isolates were active against 2 and 3 test organisms respectively (**Figure 2**).

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Table 1. Number of actinomycetes isolated from Soil samples with a screening of antibacterial activity

S. N	Soil Sample	Place of collection	Number of Colonies	Number of colonies with Inhibitory activity	
				Against 1 test organism	Against ≥2 test organism
1	A	Nuwakot (Damp Land)	5	1	1
2	B	Lagankhel (Agri Land)	14	5	3
3	C	Katunje (Agri Land)	10	3	2
4	D	Katunje (Riverbank Land)	8	3	1
5	E	Budhanilkantha (Agri Land)	13	4	3
6	F	Chapagaun (Agri Land)	19	5	4
7	G	Chapagaun (Forest Land)	8	2	2
8	H	Godawari (Damp Land)	3	0	1
9	I	Chovar (Forest Land)	3	1	0
10	J	Sundarijal (Riverbank Land)	5	4	1
11	K	Sundarijal (Forest Land)	9	1	2
12	L	Lubhu (Damp Land)	4	0	0
13	M	Sankhamul (Riverbank Land)	8	2	1
14	N	Gokarna (Forest Land)	7	2	0
15	O	Chandragiri (Forest land)	5	3	1
		Total	121	36	22

Table 2: Primary screening of effective actinomycetes against test organisms

S. N.	Actinomycetes Colony	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*S. typhi*	*S. paratyphi*	*B. subtilis*	*S. aureus*
1	A_3	-	+	-	-	-	-	+
2	B_2	-	+	-	+	+	-	-
3	B_4	-	-	+	-	-	+	+
4	B_9	+	-	+	-	-	-	+
5	C_5	+	+	-	-	-	-	+
6	C_8	-	-	-	+	+	-	-
7	D_6	-	-	+	-	-	-	+
8	E_3	+	-	-	-	-	+	+
9	E_7	+	-	+	-	-	-	-
10	E_{12}	-	+	-	+	-	-	+
11	F_1	-	-	+	+	+	-	-
12	F_2	+	-	-	-	-	+	+
13	F_{17}	-	+	+	-	-	+	-
14	F_{19}	+	+	-	-	-	-	-
15	G_1	-	+	-	+	+	-	-
16	G_3	-	-	-	-	-	+	+
17	H_4	+	-	+	-	-	-	-
18	J_2	-	-	+	-	-	+	+
19	K_1	-	+	-	-	-	+	+
20	K_6	+	-	+	-	-	-	+
21	M_3	+	+	-	-	-	-	-
22	O_4	-	-	+	+	+	-	-

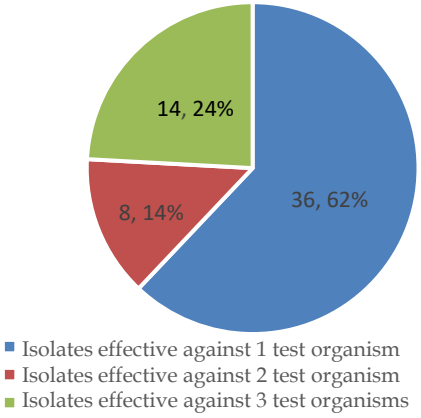

Figure 2. Pie-chart showing primary screening of actinomycetes isolates against test organisms. 36 (62%) of isolates were found to be effective in inhibiting at least one test organism. 22 (38%) of isolates were effective on inhibiting 2 or more test organisms. Of these 22 isolates 14 (24%) of them inhibited 2 test organisms while 8 (14%) effectively inhibited 3 test organisms.

Actinomycetes have broad spectrum antibacterial activity

Among test organisms, *Staphylococcus aureus* was found to be the most susceptible with susceptibility toward 12 actinomycetes isolates followed by *Klebsiella pneumoniae*, susceptible towards 10 isolates of actinomycetes. The least susceptible test organism was found to be *Salmonella paratyphi* with susceptibility towards 5 actinomycetes isolates (**Figure 3**). On secondary screening (Testing of effect of antibiotic fraction obtained through fermentation followed by extraction) against test organisms, *Staphylococcus aureus* was found to be the most effectively inhibited test organism followed by *Klebsiella pneumoniae*. *Salmonella paratyphi* was found to be the least inhibited test organism (**Table 3**).

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Table 3: Secondary Screening of Antibiotic fractions against test organisms (zone of inhibition in mm)

S.N.	Actinomycetes Colony	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*S. typhi*	*S. paratyphi*	*B. subtilis*	*S. aureus*
1	A_3		8	-	-	-	-	20
2	B_2	-	12	-	9	7	-	-
3	B_4	-	-	10	-	-	7	11
4	B_9	12	-	12	-	-	-	7
5	C_5	8	15	-	-	-	-	9
6	C_8	-	-	-	8	6	-	-
7	D_6	-	-	9	-	-	-	11
8	E_3	5	-	-	-	-	3	8
9	E_7	7	-	6	-	-	-	-
10	E_{12}	-	6	-	4	-	-	12
11	F_1		-	11	12	4	-	-
12	F_2	3		-	-	-	11	8
13	F_{17}	-	13	5	-	-	12	-
14	F_{19}	2	9	-	-	-	-	-
15	G_1	-	7	-	8	5	-	-
16	G_3	-	-	-	-	-	8	6
17	H_4	5	-	4	-	-	-	-
18	J_2	-	-	6	-	-	9	15
19	K_1	-	9	-	-	-	8	13
20	K_6	7	-	9	-	-	-	7
21	M_3	9	6	-	-	-	-	-
22	O_4	-	-	8	5	2	-	-

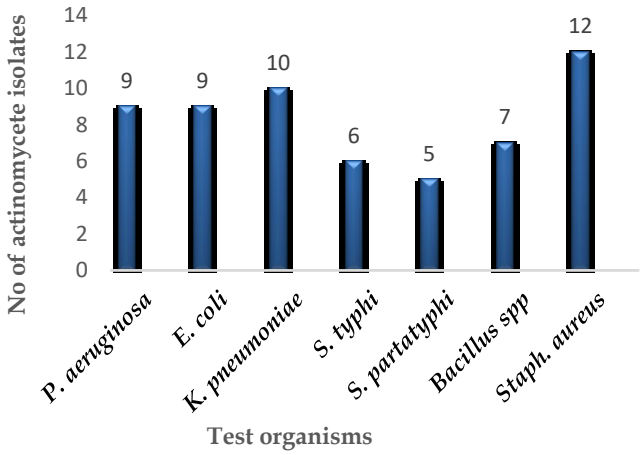

Figure 3. Bar diagram showing susceptibility of test organisms towards actinomycetes isolates. *Staphylococcus aureus* was found to be most susceptible organism toward actinomycetes with 12 isolates inhibiting its growth, followed by *Klebsiella pneumoniae* (10 isolates). *Salmonella paratyphi* was least inhibited organism with only 5 actinomycetes isolates able to inhibit its growth.

Actinomycetes have inhibitory activity against drug-resistant strains

The antibiotic fractions found to be effective against *Staphylococcus aureus* from secondary screening were tested against Methicillin Susceptible *Staphylococcus aureus* (MSSA) and Methicillin Resistant *Staphylococcus aureus* (MRSA) variants. Among 12 isolates, 10 were found to have an inhibitory effect against MSSA while only 6 were found to have an inhibitory effect against MRSA strain (**Table 4**).

Table 4: Screening Results of Effective Antibiotic Producers against MSSA and MRSA

S.N.	Actinomycetes Colony	Primary Screening		Secondary Screening (ZOI in mm)	
		MSSA	MRSA	MSSA	MRSA
1	A_3	+	+	16	10
2	B_4	+	_	7	-
3	B_9	-	_	-	-
4	C_5	+	+	9	5
5	D_6	+	+	12	8
6	E_3	-	+	6	7
7	E_{12}	+	+	8	6
8	F_2	+	-	8	-
9	G_3	+	-	6	-
10	J_2	+	-	13	-
11	K_1	+	+	9	7
12	K_6	+	-	5	-

Out of 58 effective isolates in primary screening, pH range (7.4-7.8) showed higher number of isolates followed by pH range (7.9-8.4) with 18 and 17 effective isolates respectively (**Table 5**). The least number of effective isolates were found in acidic pH range while neutral and basic pH range showed larger proportion of effective isolates.

On distribution of effective isolates based on moisture of soil, moisture range of (11-20) % showed highest number of effective isolates (17). Even though the moisture range (31-40) and (41-50) % showed high number of isolates, it has to be noted that the soil samples of river bank has high moisture due to the constant flow of water over them. This moisture range was observed only in river bank soil and the growth of actinomycetes on such soil is affected by humus and various other factors as well.

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Table 5: *Physical characterization of Soil samples*

S.N.	Soil Sample	Place/Location of collection	Color	pH	Moisture
1	A	Nuwakot (Damp Land)	Red	5.3	9 %
2	B	Lagankhel, (Agri Land)	Black-brown	7.2	32 %
3	C	Katunje (Agri Land)	Brown	8.2	20%
4	D	Katunje (River Bank Land)	Black-Brown	7.7	52%
5	E	Budhanilkantha (Agri Land)	Black	8.1	38%
6	F	Chapagaun (Agri Land)	Dark black	7.5	43%
7	G	Chapagaun (Forest Land)	Brown	6.2	12%
8	H	Godawari (Damp Land)	Brown	5.4	15%
9	I	Chovar (Forest Land)	Brown	5.2	18%
10	J	Sundarijal (River Bank Land)	Blackish	8.4	47%
11	K	Sundarijal (Forest Land)	Brown	6.4	29%
12	L	Lubhu (Damp Land)	Brown	5.8	22%
13	M	Sankhamul (River Bank Land)	Black	7.8	42%
14	N	Gokarna (Forest Land)	Black-Brown	7.6	15%
15	O	Chandragiri (Forest Land)	Reddish Brown	5.7	12%

Discussion

The soil samples undertaken for the study were of different environmental sites which include forest land, agricultural lands, river-bank land, and damp lands.

On the isolation of the Actinomycetes, it was observed that the highest number of actinomycetes isolates were isolated from 4 soil samples of agricultural land (56 out of 121 isolates i.e. 46.28%). Three soil samples from damp land were found to have 12 isolates of actinomycetes which were lowest among the soil samples classified into different ecological conditions. The higher number of isolates from agricultural and forest land can be due to the positive impact of the vegetation in the colonization of the actinomycetes.

This finding is in correspondence to the report by Ghorbani et al which states that the abundance of actinomycetes isolates decreases from irrigated cultivated land to pastures i.e., damp lands [22]. Klemmedson also reported that the actinomycetes may form the number of roots nodulated symbiotic relationship with various plants thus increasing the actinomycetes population in the lands with vegetation [23].

On the distribution of soil samples and actinomycetes isolates as per the pH value of soil, slightly alkaline pH (7.4-7.8) or moderately alkaline pH (7.9-8.4) was found to be most favorable for the proliferation of actinomycetes as 70 out of 121 isolates were obtained from these pH range (**Table 5**). Acidic pH has decreased number of actinomycetes and is not favorable for actinomycetes Agricultural lands was neutral to slightly alkaline in pH (7.2-8.2) while the pH of the forest lands was in acidic range (5.2-6.4) except for soil sample from forest land of Gokarna (pH value of 7.6) i.e. neutral pH (**Table 5**). The results of the study agree with those of Akond et al [15], Ameerah et al [24], Palanichamy et al which reported that optimum growth of actinomycetes occurs in neutral to alkaline pH [25]. However, the findings were in contrast to Kontro et al which reported 10 species of Streptomyces isolated from Finland were found to have maximum growth at pH range of (4.0-5.5) [26]. This shows that the major factor limiting actinomycetes development in forest soils is believed to be the low soil pH, as the development of most actinomycetes is favored by a neutral or slightly alkaline pH as reported by Golinska et al [27].

In this study, it was found that black colored soil harbors most of the antibiotic producing actinomycetes followed by black-brown soil in comparison to the red soil. This is in contradiction with the findings by Guo et al who reported that red soil is ideal habitat for acidophilic actinomycetes and harbors a diverse group of actinomycetes which are a promising sources of novel secondary metabolites [28].

The distribution of effective isolates as per the pH value of soil of origin showed that pH of the soil has a significant effect on the effectiveness ofthe production of the antimicrobial compounds by actinomycetes species. Also, the effective isolates were found to be from neutral to alkaline soil. These findings are in accordance with the Ameerah et al [24], Akond et al where the maximum growth of actinomycetes was reported in neutral to alkaline pH range [15]. The findings are in conformity with those of Singh et al which reported that maximum growth as well as highest antimicrobial activity by *S. sannanensis* SU118 was achieved at pH 7 while it does not exhibit any activity at pH 9 [29].

Out of 121 Actinomycetes isolates, 58 isolates were found to have inhibitory activity against at least 1 test organism i.e. 47.9% of isolates were active against at least 1 test organism. A similar result was noted in studies of Kumar et al [30] and Patel et al [12]. In contrast to the findings,

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Gurung et al reported that 34.18% of 79 isolates were active against test organisms [18] and Pandey et al found 33.96% actinomycetes isolated from the Khumbu region showed inhibitory activity against at least 1 of 5 test organisms (*Bacillus subtilis, Staphylococcus aureus, Proteus, Salmonella typhi*) [31] . Among 58 active isolates, only 22 (37.9 %) were active against 2 or more test organisms. This is in accordance with the reports of Sharma et al [32] and Salam and Rana [33]. On the contrary Devadass et al stated that only 21 out of 150 i.e. 14% of isolates from Western Ghats of Tamil Nadu were active against more than one test organism [34].

From the study *Staphylococcus aureus* was found to be most susceptible organism towards actinomycetes isolates. Also, on comparison of the zone size of inhibition against the gram-positive organism i.e. *Staphylococcus aureus* against that of the gram-negative test organisms, it can be inferred that the actinomycetes isolates were more active against gram-positive bacterium. This may be due to the difference in cell morphology of gram-positive and gram-negative cells and furthermore, the antibiotic compound could have been more functional against gram positive cell components than that of gram-negative cell components. A study carried out by Tyagi et al reported that actinomycetes isolates were highly active against *Staphylococcus aureus*, *Streptococcus, Bacillus* and *E. coli* strains. It also suggests that actinomycetes have more potent inhibitory activity against gram-positive bacteria [35].

The secondary screening of the antibacterial activity showed that out of 22 antibiotic fractions, only 8 were ineffective against gram-positive bacteria while only one fraction from isolate (G3) was ineffective against gram-negative bacteria. 13 fractions were found to be effective against both gram positive and gram-negative bacteria. These findings showed that the 9 fractions were found to have a narrow spectrum of activity while the 13 fractions were found to have a wide spectrum of inhibitory activity against the test organism. However, they cannot be confirmed as broad spectrum and narrow spectrum until they are tested multiple times with a wider range of the test organisms.

On testing of inhibitory activity of the 12 isolates active against *Staphylococcus aureus* towards drug resistant strains MRSA and MSSA, 11 were found to inhibit MSSA while 6 of them showed inhibitory activity against MRSA. These findings showed that drug-resistant strains of *Staphylococcus aureus* can be treated with the bioactive secondary metabolites from actinomycetes isolates. Sharma et al reported 6 strains of actinomycetes isolates having highly inhibitory activity against MRSA strains (MRSA-97, MRSA-67, and MRSA P-169) [32]. In a similar study Chaudhary et al reported the inhibitory activity of the 31 isolates towards *Staphylococcus aureus* among which number of them being inhibitory towards MRSA [5].

On the distribution of effective isolates as per moisture content highest number of effective isolates was found to be from soil having a moisture content of (11-20) %. The findings are inconformity with those of Mavordi et al which stated that *Pseudomonas* spp. producing the antibiotics phenazine-1-carboxylic acid were abundant in the rhizosphere of native plant species growing in non-irrigated areas adjacent to the sampled dryland wheat fields i.e. the lands with less moisture [36].

Conclusion

Soil harbor large number of actinomycetes isolates which exhibit inhibitory activity against number of bacterial strain. Acidic pH favors the growth of actinomycetes and its ability to produce antimicrobial compounds. Actinomycetes has been a constant source of antimicrobial agents and the further studies exploring the antimicrobial potential of these isolates from various unexplored areas need to be done.

Author's contribution

SB is the principal investigator, carried out all the laboratory works, also prepared the manuscript. AS is the corresponding author, an academic supervisor of the research and prepared the manuscript.

Competing Interests

The authors declare no competing or financial interests.

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Ethical Approval and Consent

Not applicable.

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