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Studies on Antimicrobial Potential of *Actinomycetes spp.* Isolated from Rhiosphere Soil against Clinical Pathogens

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ABSTRACT

Actinomycetes are the economically and biotechnologically important microorganisms which have the ability to produce wide range of antimicrobial compounds, In view of this, present study was undertaken to isolate and screen the *Actinomycetes spp.*from rhizosphere soil samples. A total of 20 *Actinomycetes* were isolated from the soil samples on Actinomycetes isolation agar and identified to *Streptomyces* and *Nocarda spp.* Further all the isolates were checked for their antimicrobial potential against 6 human pathogenic bacteria and 2 fungi. 10 isolates showed wide and prominent antibacterial and antifungal activities with zone of inhibitions ranging from 18 to 44 mm. These isolates on further detailed studies could be the good sources for development of new alternative in antimicrobials.

Keywords - *Actinomycetes*, Rhizosphere, Antimicrobial.

I. INTRODUCTION

Actinomycetes are aerobic, Gram-positive bacteria. They are one of the major groups of soil population and are very widely distributed (Kuster, 1968). The number and types of *Actinomycetes* present in a particular soil would be greatly influenced by geographical location search as soil temperature, soil type, soil pH, organic matter content, cultivation, aeration and moisture content (Arifuzzaman *et al.,* 2010). *Actinomycetes* are Gram-positive mycelium-forming soil bacteria represent a ubiquitous group of microbes that are the most economically and biotechnologically valuable prokaryotes. They have different characteristics from those of terrestrial counterparts and therefore might produce different types of bioactive compounds (Sharma and Shah, 2014).

Soil microorganisms provide an excellent resource for the isolation and identification of therapeutically important products. Among them, *Actinomycetes* are an important group of filamentous, Gram-positive bacteria producing antibiotics of agricultural and medicinal importance. *Streptomyces spp.* rank first and cover around 80% of total antibiotics (Berdy, 2005). Though there are number of antibiotics, the need to search for new and efficient antibiotic. Producing strains keeps rising due to the emergence of drug resistant pathogens (Wise, 2008). Particularly, the incidences of infections by opportunistic fungi are increasing, especially in patient whose immune

system are compromised by AIDS, cancer, diabetes, age and other causes. Many antifungal compounds have been identified, but safe and effective antifungal drugs have not yet been developed because of high degree of similarity between fungi and mammalian cells (Berdy, 1989).

Streptomyces are valuable resources for novel products like antifungals, antivirals, antitumoral, anti-hypersensitive, immunosuppressants and especially antibiotics (Awasthi *et al.,* 2014). The nature of the active agent of the antibiotics produced by *Actinomycetes* depends upon the species; frequently upon the strain and the condition of cultivation (Waksman *et al.,* 2010). The time of discovery of antibiotics, the emergence antibiotic resistant bacteria have been a major problem. Also, there is a rapid emergence of drug resistant strain of the pathogen that the rate of discovery of new drug and antibiotics (Budhathoki and Shreshta, 2020).

The microbes in rhizosphere help plants in growth-promotion and yield. *Actinomycetes* are one of the major components of rhizosphere microbial populations and are useful in soil nutrient cycling as well as plant growth-promotion (PGP). *Actinomycetes* produce secondary metabolites such as lytic enzymes, PGP substances and antibiotics. The *Actinomycetes*, mainly those belonging to *Streptomyces spp.,* make up an important group of soil microbes. *Streptomyces* are abundant in soil and help in the degradation of complex molecules to simple molecules for plant growth and development. These are also reported to decompose organic matter, promote plant growth and control plant pathogens. 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 (Budhathoki and Shreshta, 2020). Hence, screening of anti-microbial activity of *Actinomycetes* and study of their antimicrobial action against pathogen is an important process for the discovery of an antibiotic.

II. METHODS AND MATERIAL

Collection of Samples

A total of 20 rhizosphere soil samples were collected from the plants. The soil was collected from the depth of 10–15 cm near the roots using sterile spatula and collected in clear dry sterile polythene bags. All the samples were transported to laboratory and stored in the refrigerator at 4°C for further use.

Preparation of soil samples

Soil samples 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 (Akond *et al.,* 2004 and Heng *et al.,* 2011).

Isolation & Identification of *Actinomycetes*

Isolation and Identification of potent *Actinomycetes* isolates was carried out by morphological and biochemical characteristics.

Study of Antimicrobial Activity

Screening of Antimicrobial activity of *Actinomycetes* isolate against test organism was done by two methods

1) Perpendicular Streak Method
2) Agar Well Diffusion Method.

Perpendicular Streak Method

All the *Actinomycetes* isolates obtained were tested for their antimicrobial activities against test organism. Screening for antimicrobial activity of pure isolates was determined by perpendicular streak method using Nutrient agar medium, The isolates were streak as a straight line across diameter on Nutrient agar plates and incubated at 28°C for 5 days, the test organism namely, *S. aureus, E. coli, P. aeruginosa, S. typhi, B. subtilis, C. albicans* and *A. niger* culture were streaked at right angle, but not touching the streaked isolate and incubated at 37°C for 24 hours. Clear zone

formation between the antibiotic producing isolates and test organism was considered positive for antibiotic production (Muleta and Assefa, 2018).

Agar Well Diffusion Method

Agar well diffusion method was employed for secondary screening of inhibitory action against test organism, The isolates of *Actinomycetes* are inoculated in the Trypton Soy Broth and incubated at 28°C for 5 days after incubation the broth was centrifuged at 10,000 rpm for 20 minutes. The cell free supernatant obtained was collected. Meanwhile the pre-inoculated test microorganisms as *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus, Candida albicans* and *Aspergillus niger* was inoculated onto Nutrient broth was removed from incubator. The cultures of test clinical pathogens were inoculated on sterilized Muller Hinton agar plates by sterile cotton swab uniformly. With the sterile cork borer, a well of 5 mm was prepared into the agar media. The cell free supernatant (CFS) from *Actinomycetes* isolates were then put into each well. The plates were then incubated for 24 hrs at 37°C for bacteria and at room temperature for 48 hrs for fungi. After incubation the plates were observed for development of zone of inhibition around the wells.

III. RESULTS AND DISCUSSION

In the present study, totally 20 *Actinomycetes* strain were isolated from soil samples of different plants based on the gram staining and colony morphology. All the isolates were found to be positive in gram staining and colony morphology. All the isolates were recorded. According to these results emphasized that the *Actinomycetes* isolates is related to a group of *Streptomycetes,* and *Nocardia spp.* based on Bergey's Manuel of Systemic Bacteriology.

In the present study, 90% of *Actinomycetes* isolated were belongs to *Streptomyces spp.* and 10 % were found to be of *Nocardia spp.* This is in similar with findings of Kumar *et al.,* (2012) who also isolated 72.7% *Streptomyces spp.* and 13.6% of *Norcardia spp.* This is also in line with previous results of Kieser *et al.*, (2000) who also reported *Streptomyces* frequently and isolated 80% of total antibiotics producers are from this genus compared to others.

The isolates were screened for their inhibitory activity against the pathogenic bacteria. Both the primary and secondary screening methods were used to screen the *Actinomycetes* for antimicrobial activity. The identified isolates were then subjected to primary screening as present in Table No.1 out of 20 isolates, 11 isolates showed antimicrobial activity against test organism during primary screening by the perpendicular streaking method.

It was found that few isolates showed the antimicrobial activities against test microorganisms in primary screening as compared to secondary screening as not much prominent activities were not recorded. The present findings was also supported by study of Pandey *et al.,* (2004) and Bushell (1993) who reported some of the *Actinomycetes* isolates in primary screening did not show any activity but in secondary screening some showed little to improved activity.

Among 20 isolates, 16 isolates showed zone of inhibition against test organism in the secondary screening as shown in Fig. 2–9. *E. coli* showed complete resistance towards the isolates S4, S5, S7, S13 and S15. It was found that *E. coli* was more sensitive towards isolate S14 (35 mm), S16 (33 mm), S19 (31 mm) whereas the least antimicrobial activity was given by isolate S2 and S6 forming a zone of inhibition of 14 mm respectively.

S. aureus was found to be sensitive towards all isolates. Maximum zone of inhibition was given by S17 (35 mm) and minimum was given by S9 (9 mm)

P. aeruginosa was found to be resistance towards the isolates S2 and S10. It was found that *P. aeruginosa* was more sensitive towards isolates S3 (35 mm), S4 (37 mm), S5 (38 mm), S7 (34 mm) and S8 (39 mm), whereas the least antimicrobial activity was given by isolate S11, S17 and S20 forming a zone of inhibition 19 mm, 19 mm and 12 mm respectively

S. typhi showed complete resistant towards to isolate S6, S8, S10, S11 and S 14. It was found that salmonella was more sensitive towards isolates S13 (39 mm), S15 (30 mm), S16 (39 mm) and S18 (35 mm), whereas the least anibicrobial activity was given by isolates S2 and S5 forming a zone of inhibition of 15 mm and 12 mm respectively

K. pneumoniae was found to be sensitive towards all isolates. Maximum zone of inhibition was given by S6 (33mm), and S10 (37mm) and minimum was given by S3 (16 mm).

B. subtilis showed complete resistance towards the isolate S13 and S15. It was found that *B. subtilis* was more sensitive towards isolate S16 (32 mm), S12 (30 mm), S17 (40 mm) and S20 (30 mm), whereas the least antimicrobial activity was given by isolate S14 forming a zone of inhibition of 23 mm respectively.

C. albicans was found to be sensitive towards all isolates. Maximum zone of inhibition was given by S12 (43 mm), S14 (41mm), S15 (42 mm), S16 (44mm), S17 (41 mm) and S18 (40 mm) and minimum was given by S11 and S13 (22 mm).

A. niger was found to be sensitive towards all isolates. Maximum zone of inhibition was given by S12 (38 mm). S13 (35mm) and S14 (36 mm) and minimum was given by S11, S15, S17 (20 mm)

This finding is in correspondence to the report by Mulcta and Assefa (2018). They found in their study that the antimicrobial compounds obtained from AAUBA13 crude extract has an antibacterial activity and also and antifungal activity. The data in general showed that the antimicrobial compounds obtained from AAUBA13 demonstrate broad spectrum and remarkable antimicrobial activity against bacterial and *C. albicans* ATCC62376. The similar results were recorded by Kumar *et al.,* (2012) who also isolated *Streptomyces and Nocardia spp.* along with other species and reported promising activity against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa,* Budhatoki and Shrestha (2020), also isolated 58 *Actinomycets* from soil samples which found to have inhibitory activity against at least one test organism like *P. aeruginosa, E. coli, K. pneumoniae, S. typhi, S. aureus* and *B. subtilis.* Gurung *et al.,* (2009) reported that 34.18% of isolates and Pandey *et al.,* (2004) reported 33;.96% of *Actinomycetes* isolates showed inhibitory activity against atleast 5 test organisms.

The genera *Streptomyces* and *Nocardia* are well known for antifungal metabolites (Berdy, 2005; Mikami, 2007). The present study also showed the isolates were showed prominent antifungal activities against *C. albicans* and *A. niger.* This is in accordance with the Kavitha *et al.,* (2010) who have also isolated *Streptomyces* and *Nocordia spp.* which showed good antifungal activities against *C. albicans* and *A. niger.* Kathiresan *et al.,* (2005) also reported *Streptomyces spp.* showed good antifungal activity against filamentous fungi.

Actinomycetes isolates recovered from rhizosphere soil sample showed the potential to produce antimicrobial bioactive compounds. It is also suggested that the other isolates should be further processed to realize their antibiotic property on different test microorganism

Table 1 : Primary Screening of Antimicrobial activity of Isolate by Perpendicular Streak Method

Characteristics	Isolates																			
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
E. coli	-	-	+	-	-	+	-	-	+	-	+	-	+	-	-	+	+	-	+	-
S. aureus	+	+	-	-	+	-	-	-	-	+	-	-	+	+	-	-	+	-	-	-
P. aeruginosa	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	-	-	+	+
S. typhi	+	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	-	+	-
B. subtilis	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-
K. pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

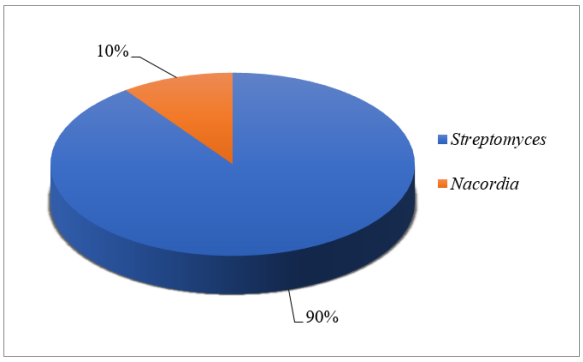
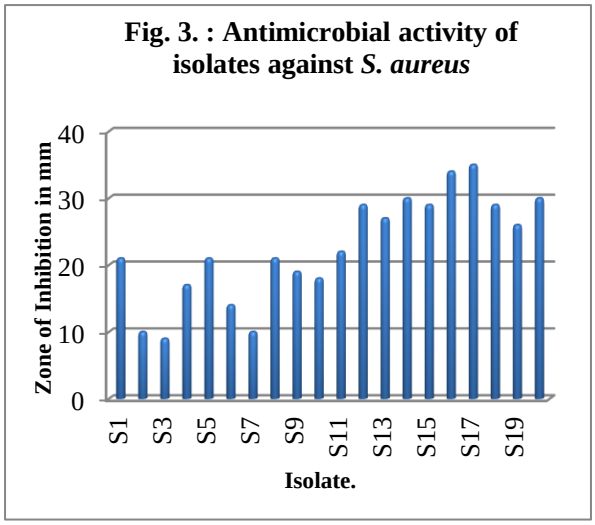
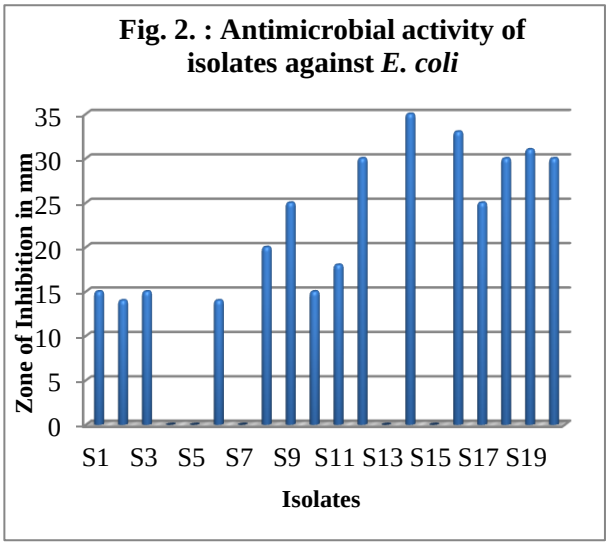
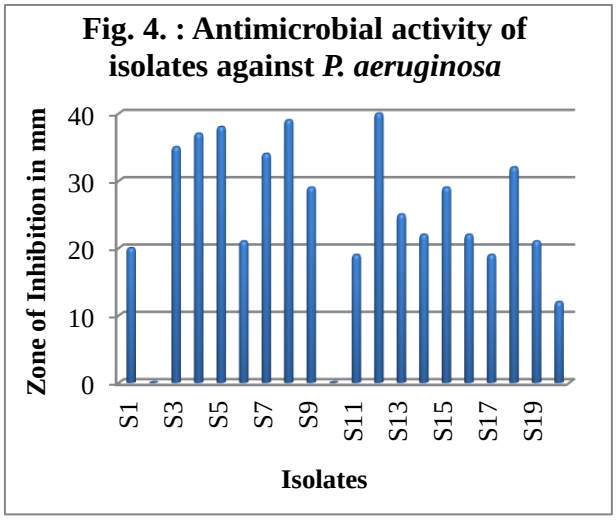


Fig. 1 : *Actinomycetes* obtained form soil sample







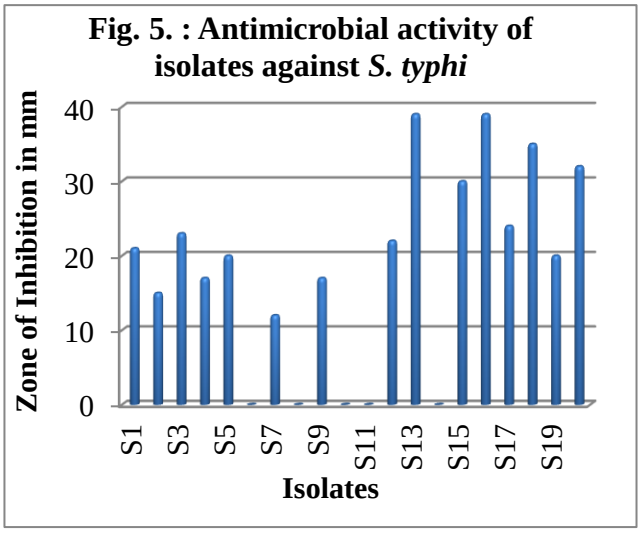


Fig. 5. : Antimicrobial activity of isolates against *S. typhi*

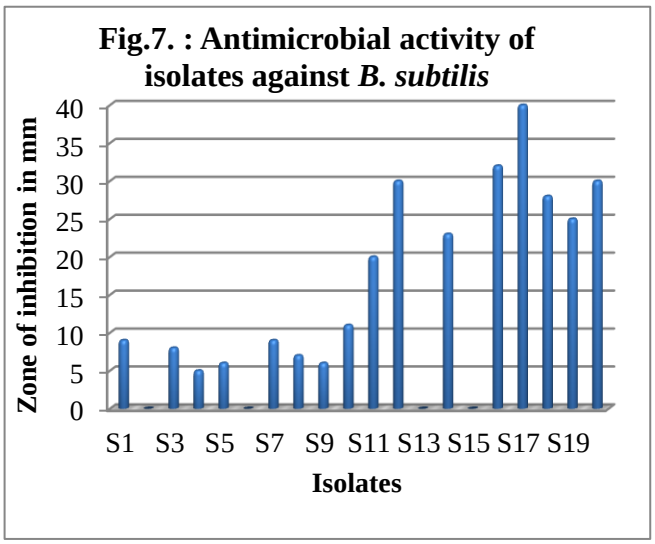


Fig.7. : Antimicrobial activity of isolates against *B. subtilis*

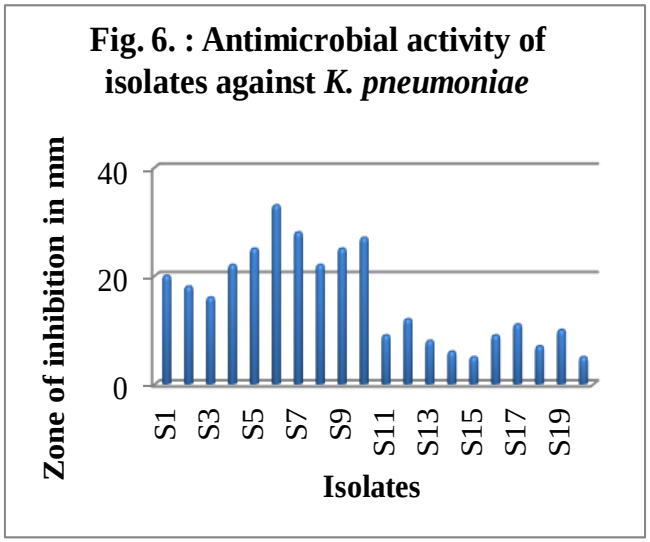


Fig. 6. : Antimicrobial activity of isolates against *K. pneumoniae*

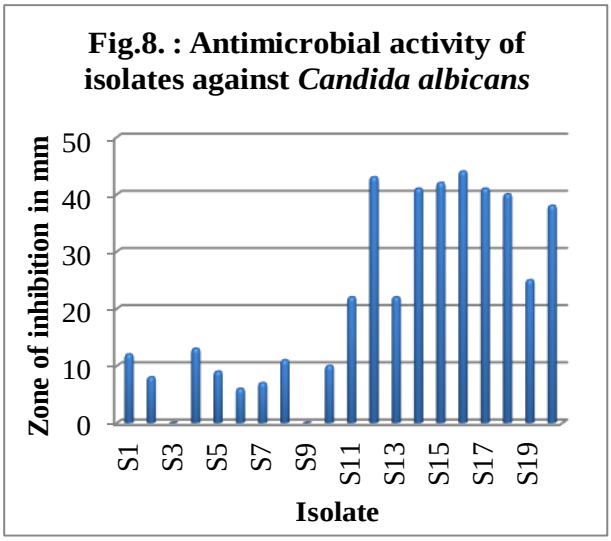
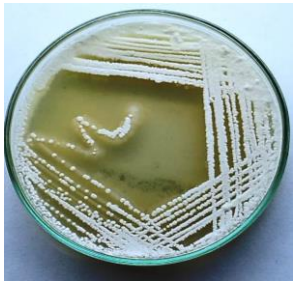
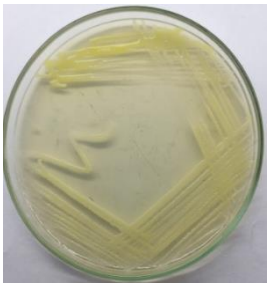
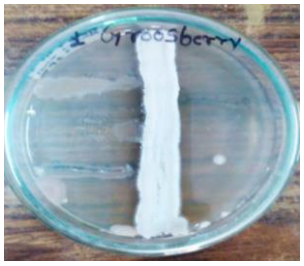


Fig.8. : Antimicrobial activity of isolates against *Candida albicans*







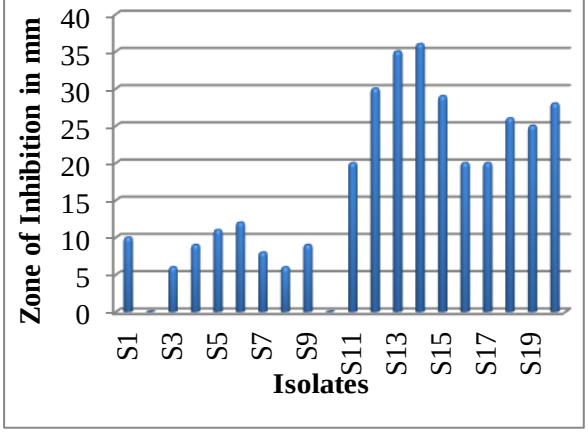
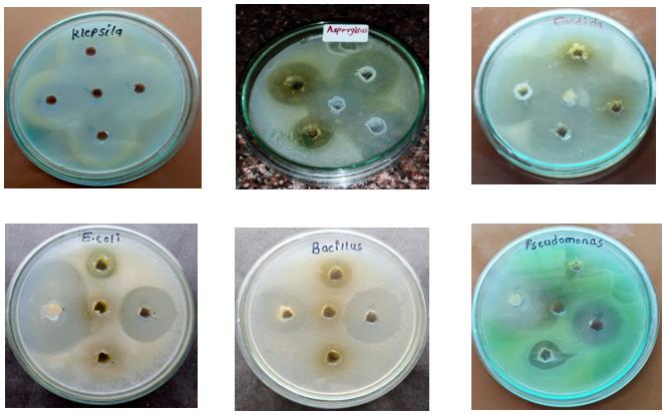


Fig.9. : Antimicrobial activity of isolates against *A. niger*

Isolation and Identification of Actinomycetes



Primary Screening of Actinomycetes for Antimicrobial Activity

IV. CONCLUSION

The finding of the present study showed that Actinomycetes isolates were belongs to the Streptomyces and Nocordia spp. These isolates were showed prominent antibacterial and antifungal potential against tested clinical pathogens which showed the ability of these isolates for antimicrobial metabolites production. Thus, further detailed study on isolation, purification and optimization of these prominent rhizosphere Actinomycetes would be beneficial in the production of new antimicrobial compounds.

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