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Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town, North West Ethiopia

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PEER REVIEW

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Comments

This is a good study in which the authors tested different soil samples at different ecological areas of Gondar. There is no report about this industrially and biotechnologically important actinomycetes for the production of secondary metabolites like antibiotics in the study areas before.

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ABSTRACT

Objective: To isolate and screen antibiotic producing actinomycetes from potential soil samples of Gondar town, Ethiopia.

Methods: Fifteen soil samples were collected, serially diluted and spread on starch casein and oat meal agar supplemented with amoxicillin and cyclohexamide for inhibition of bacteria and fungi, respectively. Cross streak method was used to check antagonistic activity of isolated actinomycetes against test organisms. Solid state fermentation and crude extraction were used for the production of antibiotics from isolates. Agar well diffusion was used for antimicrobial activity of crude extracts against test organisms.

Results: Three isolates (Ab18, Ab28 and Ab43) have been shown high antagonistic activity during primary screening. Inhibition zones obtained from crude extracts showed significance differences when compared with standard antibiotics tested against test organisms (P<0.05). Inhibition zone of crude extracts from isolate Ab18 against *Klebsiella pneumonia* ATCC7000603 and *Escherichia coli* ATCC25922 were (14±1) mm and (35±1) mm, respectively which were strong active when compared to amoxicillin (0 mm) and tetracycline [(13±1) mm for *Klebsiella pneumonia* ATCC7000603 and (33±1) mm for *Escherichia coli* ATCC25922]. Crude extracts from isolate Ab18 showed (20±1) mm and (15±1) mm inhibition zones against methicillin resistant *Staphylococcus aureus* strains 2 (MRSA2) and MRSA4, respectively. Crude extract from isolate Ab43 has shown high antimicrobial activity (18±1) mm against MRSA2 and MRSA4.

Conclusions: There was not any scientific report on soil actinomycetes producing antibiotic in the study areas. Therefore, isolation and screening of actinomycetes from such areas in optimum condition may contribute the discovery of new antibiotics. Potent antibiotics from these actinomycetes could contribute a lot to fight against antibiotic resistant pathogens. Selected isolates have been shown strong antimicrobial activity against resistant pathogens. Further purification, structural elucidation and characterization are recommended to know the quality, novelty and commercial value of these antibiotics.

KEYWORDS Actinomycetes, Antibiotics, Gondar town, Isolation, Pathogens, Screening

1. Introduction

Secondary metabolites are produced by some organisms such as bacteria, fungi, plants, actinomycetes and so forth. Among the various groups of organisms that have the capacity to produce such metabolites, the actinomycetes occupy a prominent place^[1-3]. Actinomycetes are prokaryotes of Gram-positive bacteria but are distinguished from other bacteria by their morphology, DNA rich in guanine plus cytosine (G+C) and nucleic acid sequencing

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and pairing studies. They are characterized by having a high G+C content (>55%) in their DNA[4-6].

Actinomycetes are of universal occurrence in nature and are widely distributed in natural and man-made environments. They are found in large numbers in soils, fresh waters, lake, river bottoms, manures, composts and dust as well as on plant residues and food products. However, the diversity and distribution of actinomycetes that produce secondary metabolites can be determined by different physical, chemical and geographical factors^[5,6].

Actinomycetes provide many important bioactive substances that have high commercial value. Their ability to produce a variety of bioactive substances has been utilized in a comprehensive series of researches in numerous institutional and industrial laboratories. This has resulted in the isolation of certain agents, which have found application in combating a variety of human infections^[7]. That is why more than 70% of naturally occurring antibiotics have been isolated from different genus of actinomycetes^[8]. Out of these different genus, *Streptomyces* is the largest genus known for the production of many secondary metabolites^[9], which have different biological activities, such as antibacterial, antifungal, antiparasitic, antitumor, anticancer and immunosuppressive actions^[1,10,11].

Some antibiotics like penicillin, erythromycin, and methicillin which used to be one-time effective treatment against infectious diseases^[12,13], are now less effective because bacteria have become more resistant to such antibiotics. Antibiotic resistant pathogens such as methicillin and vancomycin resistant strains of *Staphylococcus aureus* (*S. aureus*) and others cause an enormous threat to the treatment of serious infections. To avoid this happening, immediate replacement of the existing antibiotic is necessary^[13], and the development of novel drugs against drug resistant pathogens is significant for today.

Thus, finding and producing new antibiotics as well as using combined antibiotic therapy have been shown to delay the emergency of microbial resistance and can also produce desirable synergistic effects in the treatment of microbial infection. Antibiotic synergisms between known antibiotics and bioactive extracts are a novel concept and have an important activity against pathogens and host cells^[14].

Research in finding newer antibiotics and increasing productivity of such agents has been a very important activity^[3,7]. This is because some important drugs are expensive and/or have side effect to the host, some microbes have no successful antibiotics and others are developing multidrug resistance. These situation requires more attention to find solutions by searching and producing new and effective antibiotics from microbes like actinomycetes. However, there is no such scientific report on antibiotic producing actinomycetes from soil samples collected in Gondar, Ethiopia. Therefore, the objective of the present study was to isolate and screen antibiotic producing actinomycetes from soil samples in Gondar. The outcome of this finding may be important to give direction for researchers and for future treatment of multidrug resistant human pathogens.

2. Materials and methods

2.1. Study area and period

The study area was located at Northwest part of Ethiopia, Gondar. According to 2008 Ethiopian Statistical Agency Report, Gondar town has 20 Kebeles with a population of 231977. Kebele 16 sites (such as Johannes, Gibirina and Condominium) and Kebele 18 sites (such as Tewodros Campus and Taxi Mazoria) were randomLy selected for study. The study was carried out from September 2011 to August 2012.

2.2. Sampling and isolation of actinomycetes

Samples were collected from waste disposal (at Taxi Mazoria, Johannes, Gibirina and Condominium) and rhizosphere (at Tewodros Campus) soil areas. During the study, 15 soil samples were collected aseptically from 5 sites at different depth (5, 8 and 11 cm) of the soil using standard methods^[15,16]. The collected samples were transferred to research laboratory of microbiology, Department of Biology in Tewodros campus where the entire research work was carried out. The soil samples were sieved through 250 µm pore size sieve (United Kingdom). From each sample, 1 g of soil sample was then added in different test tubes containing 10 mL physiological saline (NaCl, 8.5 g/L) and shaken well using vortex mixer. These test tubes were considered as stock cultures for different soil sample sites.

From the stock cultures, a volume of 1 mL was transferred aseptically and added to a test tube containing 9 mL of sterile physiological saline and mixed well. From this test tube, 1 mL of aliquot was again transferred and mixed with another 9 mL of sterile physiological saline to make 10^{-2} dilution factor. Similarly, dilutions up to 10^{-5} were made using serial dilution technique for all soil samples. A volume of 1 mL of suspension from 10^{-4} and 10^{-5} serially diluted tubes were taken and spread evenly with sterile L–shaped glass rod over the surface of sterile starch casein and oat meal agar plates aseptically using spread plating technique. Amoxicillin (20 µg/mL) and cyclohexamide (25 µg/mL) were added in both media to inhibit bacterial and fungal

contamination, respectively. The plates were incubated aerobically at 37 °C up to 7 d and observed intermittently during incubation^[17]. After incubation, actinomycetes on the plates were identified based on color, dryness, rough, convex colony. The identified colonies were purified by repeated streak plate method^[15,18].

After isolation of the pure colonies, each colony was further identified on the basis of its earthy like smell, colonial morphology, colour of hyphae and the presence or absence of aerial and substrate mycelium. Then, selected and identified colonies of actinomycetes were transferred from the plate to starch casein agar slant and incubated at 37 °C for growth. After incubation, the slants containing pure isolated actinomycetes were preserved at -4 °C and maintained longer by periodic subculture on starch casein agar^[18].

2.3. Primary screening

Actinomycetes isolated and identified from different soil samples were screened for their antimicrobial spectrum. The test bacteria used for primary screening were S. aureus ATCC2923, Psedomonas aeruginosa ATCC27857 (P. aeruginosa), Escherichia coli ATCC25922 (E. coli), Klebsiella pneumonia ATCC7000603 (K. pneumonia) and Salmonella typhi ATCC9289 (S. typhi). Anti-fungal activity of actinomycetes was determined using Saccharomyces cerevisiae (S. cerevisiae) as test organism. Activities were assessed using nutrient agar (India) for bacteria and potato dextrose agar (India) for fungi. Each plate was streaked with each isolate at the centre of a plate and incubated at 37 °C for 7 d. Then, fresh subcultured test organisms were streaked perpendicular to the actinomycete isolate^[5,19]. Then the plates were incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi. After incubation, the zone of inhibition was measured and recorded.

2.4. Fermentation and extraction of crude extracts

Based on the zone of inhibition in primary screening, isolates (designated as Ab18, Ab28 and Ab43) that have potential antimicrobial activity were selected for solid state fermentation and extraction, and then the crude extracts were assessed following agar well diffusion methods^[20]. The promising cultures of actinomycete isolates were grown in starch casein broth (200 mL) at 37 °C for 7 d. Lastly, 10% of cultured broth was inoculated into sterilize Erlenmeyer flask containing natural media (40 g wheat grain and 20 mL milk) on thermostat water bath at 37 °C for 7 d.

To concentrate the antimicrobial metabolite produced from Ab18, Ab28 and Ab43 isolates, equal volume of ethyl acetate (200 mL) was added in each solid state fermented cultures for 1 h in thermostat water bath shaker at 37 °C[21]. Then the ethyl acetate containing active metabolite was separated from the solid residue with Whatman No.1 filter paper and extracts were concentrated using rota vapour. The crude extracts obtained from each isolates were dissolved in ethyl acetate (76 mg/mL) and used as stock concentration for determination of antimicrobial activity against test pathogens using ethyl acetate as a control.

The wells (6 mm diameter) were cut using a sterile cork borer on Muller Hinton agar (MHA, India) and potato dextrose agar (India). Twenty four hours young culture of *S. aureus* ATCC2923, methicillin resistant *S. aureus* (clinical isolates), *E. coli* ATCC25922, *S. typhi* ATCC9289, *K. pneumonia* ATCC7000603, *S. boydi* ATCC9289) and 48 h young culture of *Candia albicans* (*C. albicans*, clinical isolates) were swabbed with sterilized cotton swab on the surface of prepared Muller Hinton agar for bacteria and potato dextrose agar for fungi. Sixty micro litres of dissolved crude extract was loaded into each well and left for 30 min until the metabolite was diffused. Then the plates were incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi. After incubation, the zone of inhibitions were measured and recorded.

2.5. Data analysis

The collected and recorded data were analysed using SPSS 16 Version software. The different inhibition zone measurements in triplicate were compared by performing One–way ANOVA ranked with Duncan's multiple range tests with descriptive analysis type on different isolates against different test pathogens. All statistical results with P<0.05 were considered to be statistically significant.

3. Results

3.1. Sampling and isolation of actinomycetes

From a total of 15 soil samples, 30 different actinomycete isolates were obtained at different depth of the soil. Of these 30 isolates, two (6.7%) were isolated from near cooking (kitchen) house soil, ten (33.3%) from breeding area and three (10%) from house waste disposal area of Kebele 16. On the other hand, one (3.3%) was isolated from rhizosphere soil and fourteen (46.7%) were isolated from near industrial waste disposal area of Kebele 18 (Table 1).

3.2. Screening of isolated actinomycetes for their antimicrobial activities

3.2.1. Primary screening

As the results of primary screening, eight (26.7%) actinomycete isolates were showed antimicrobial activity against one or more test bacteria and fungus. Of these eight

isolates, five (Ab24, Ab28, Ab41, Ab43 and Ab44) which account for 62.5% from industrial waste disposal area, two (Ab13 and Ab18) which account for 25% from cattle breeding area and one (Ab5) which account for 12.5% from near kitchen house were obtained.

Among a total of 8 isolates showing antimicrobial activities, three (37.5%) isolates which was designated as Ab24, Ab41 and Ab44, have been shown antimicrobial activity against Gram negative bacteria only. In this study, one (12.5%) isolate, Ab18, was active against both Gram positive and Gram negative bacteria. Moreover, four (50%) isolates (Ab5, Ab13, Ab28 and Ab43) were active against Gram negative, Gram positive and fungus. From the total 8 isolates, five (62.5%) have been shown antagonistic activity against *S. aureus* ATCC29213, *E. coli* ATCC25922, *S. typhi* ATCC9289 and *K. pneumonia* ATCC7000603. In addition to the above, four (50%) isolates out of 8 were shown antagonistic activity against *S. cerevisiae*. However, *P. aeruginosa* ATCC27853 was resistant against all isolates.

There was high significant difference (P < 0.05) among

antagonistic activity of isolates against *S. aureus*. The most promising isolate against *S. aureus* was the Ab43 isolate (25 \pm 1) mm when compared to other isolates. Isolate Ab28 (20 \pm 1) mm, Ab18 (15 \pm 1) mm, Ab13 (13 \pm 1) mm and Ab5 (10 \pm 2) mm were the second, third, fourth and fifth potent isolates against *S. aureus*, respectively. However, *S. aureus* showed resistant against Ab24, Ab41 and Ab44 isolates.

The three isolates (Ab13, Ab24 and Ab41) did not show any antimicrobial activity against *E. coli*. However, the isolate Ab43 (30±2) mm showed the highest antagonistic activity against *E. coli* when compared to others. The isolates that showed the second, third and fourth antagonistic activities against *E. coli* were Ab28 (16±2) mm), Ab44 (13±1) mm and Ab5 (8±1) mm, respectively. As the result indicated in Table 2, isolate Ab18 (15±1) mm shows antagonistic activity between isolate Ab44 (13±1) mm and Ab28 (16±2) mm against *E. coli*.

S. typhi have shown high resistance against antibiotics of the three isolates (Ab13, Ab24 and Ab44). However, isolate Ab43 (32±2) mm showed the first most antagonistic activity

Table 1

Description of samples collected from different sites of Gondar town and isolated actinomycete.

| Sample sites | Districts | Kebele | Specific soil sample area | Soil depth (cm) | Numbers of isolates and their code |
|--------------|-----------------|--------|---------------------------|-----------------|--|
| S1 | Tewodros Campus | 18 | Rhizosphere | 11 | Ab1 |
| | | | | 8 | 0 |
| | | | | 5 | 0 |
| S2 | Johannes | 16 | Cooking house | 11 | Ab4 |
| | | | | 8 | Ab5 |
| | | | | 5 | 0 |
| S3 | Gibirina | 16 | Cattle breeding area | 11 | Ab6, Ab7, Ab8, Ab9 |
| | | | | 8 | Ab11, Ab12, Ab13, Ab15 |
| | | | | 5 | Ab16, Ab18 |
| | | | | 11 | Ab20, Ab21 |
| S4 | Condominium | 16 | House waste disposal area | 8 | Ab22 |
| | | | | 5 | 0 |
| S5 | Taxi Mazoria | 18 | Industrial waste disposal | 11 | Ab24, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31 |
| | | | | 8 | Ab32, Ab33, Ab34, Ab37 |
| | | | area | 5 | Ab41, Ab43, Ab44 |
| Total | | | | | 30 |

Ab: Designation for each isolated actinomycetes.

Table 2

Comparison of the antagonistic activity of isolates against test organisms during primary screening.

| Inglatas | Inhibition zones (mm) | | | | | | | |
|----------|-----------------------|---------------|-------------------|-----------------------|-------------------|------------------|--|--|
| isolates | S. aureus | P. aeruginosa | E. coli | S. typhi | K. pneumonia | S. cerevisiae | | |
| Ab5 | 10 ± 2^{b} | 0 | $8\pm1^{\rm b}$ | 7±1 ^b | 0 ± 0^{a} | $13\pm1^{\circ}$ | | |
| Ab13 | $13\pm1^{\circ}$ | 0 | 0 ± 0^{a} | 0 ± 0^{a} | 12 ± 1^{b} | 10 ± 1^{b} | | |
| Ab18 | 15 ± 1^{d} | 0 | $15\pm1^{\rm cd}$ | $14\pm1^{\circ}$ | 20 ± 2^{d} | 0 ± 0^{a} | | |
| Ab24 | 0 ± 0^{a} | 0 | 0 ± 0^{a} | 0 ± 0^{a} | $15\pm1^{\circ}$ | 0 ± 0^{a} | | |
| Ab28 | $20\pm1^{\rm e}$ | 0 | 16 ± 2^{d} | 25 ± 1^{d} | $15\pm 2^{\circ}$ | $15\pm2^{\circ}$ | | |
| Ab41 | 0 ± 0^{a} | 0 | 0 ± 0^{a} | $8\pm 2^{\mathrm{b}}$ | 0 ± 0^{a} | 0 ± 0^{a} | | |
| Ab43 | 25 ± 1^{f} | 0 | 30 ± 2^{e} | 32 ± 2^{e} | 20 ± 1^{d} | $15\pm2^{\circ}$ | | |
| Ab44 | 0 ± 0^{a} | 0 | $13\pm1^{\circ}$ | 0 ± 0^{a} | 0 ± 0^{a} | 0 ± 0^{a} | | |

Values are means±SD. The outcomes not sharing a common superscript letter in the same column are significantly different at P<0.05.

against *S. typhi*. The second and third potent isolates against *S. typhi* were Ab28 (25±1) mm and Ab18 (14±1) mm, respectively. Two isolates, namely Ab41 (8±2) mm and Ab5 (7 ±1) mm have shown the fourth potent antagonistic activity against *S. typhi*.

Isolates Ab18 (20±2) mm and Ab43 (20±1) mm have shown significantly (P<0.05) highest antagonistic activity against K. *pneumonia*. On the other hand, isolate Ab24 (15±1 mm) and Ab28 (15±1 mm) have shown the second antagonistic activity against K. *pneumonia*. The only isolate that showed the third antagonistic activity against K. *pneumonia* was Ab13 (12±1) mm. However, K. *pneumonia* showed resistance against Ab5, Ab41 and Ab44 isolates.

The three isolates, such as Ab28 (15 ± 2) mm, Ab43 (15 ± 2) mm and Ab5 (13 ± 1) mm have shown the first antagonistic activity against *S. cerevisiae* strain when compared to other isolates. Isolate Ab13 (10 ± 1) mm was the second potent isolate that showed antimicrobial activity against this fungus. However, *S. cerevisiae* has shown resistance against four isolates (Ab18, Ab24, Ab41 and Ab44) (Table 2).

3.2.2. Secondary screening

Except isolate Ab28, crude extracts of promising isolates have been shown to have statistically significant activity (P < 0.05) against most of test organisms during secondary screening process. Crude extracts from isolates (except isolate Ab28) showed high antimicrobial activity against S. aureus in comparison with standard amoxicillin. Crude extract from isolate Ab18 (35 \pm 1) mm have shown higher activity against *E*. coli compared to Ab28 (0 mm), Ab43 (25±1) mm, amoxicillin (0 mm) and tetracycline (33±1) mm. S. typhi (25±2) mm and K. pneumonia (14±1) mm were more sensitive to crude extract of isolate Ab18. Therefore, there was a statistically significant (P < 0.05) antimicrobial activity of crude extract of isolate Ab18 in comparison with that of amoxicillin and tetracycline (Table 3). Crude extract of isolate Ab18 (15±1) mm have shown better activity in comparison with other extracts against C. albicans but less than that of cyclohexamide (25 ± 1) mm. Crude extract of isolate Ab28 (12±1) mm was the third active extract against this fungus. However, C. albicans was resistant to crude extract of isolate Ab43 (Table 3).

Out of the three isolates with greater antimicrobial activity in primary screening, the promising isolates (except isolate Ab28) also showed inhibitory action against clinical isolates of methicillin resistant *S. aureus* strains (MRSAs) during secondary screening. The crude extracts have shown promising and encouraging antimicrobial activities against two MRSA strains when compared to the standard antibiotics of penicillin, methicillin and vancomycin (Table 4).

Table 3

Agar well diffusion assay of ethyl acetate extract of potential isolates against test organisms.

| Crude extracts and | | | Zone of inl | hibitions (mn | n) | |
|----------------------|------------------|--------------------|------------------|---------------|-------------------|------------------|
| standard antibiotics | SA | EC | ST | KP | SB | CA |
| Ab18 | $30\pm1^{\circ}$ | 35 ± 1^{d} | $25\pm2^{\circ}$ | 14 ± 1^{c} | 37±1 [°] | $15\pm1^{\circ}$ |
| Ab28 | 0 ± 0^{a} | 0 ± 0^{a} | 0 ± 0^{a} | 0 ± 0^{a} | 15 ± 1^{a} | 12 ± 1^{b} |
| Ab43 | 34 ± 1^{d} | 25 ± 1^{b} | 17 ± 1^{b} | 0 ± 0^{a} | 30 ± 1^{b} | 0 ± 0^{a} |
| Ab18 & 43 | 25 ± 1^{b} | 0 ± 0^{a} | 15 ± 1^{b} | 12 ± 1^{b} | 40 ± 1^{d} | 0 ± 0^{a} |
| AMC30 | 25 ± 1^{b} | 0 ± 0^{a} | 15 ± 1^{b} | 0 ± 0^{a} | 45 ± 1^{e} | - |
| TE30 | $40\pm1^{\rm e}$ | $33 \pm 1^{\circ}$ | $26\pm1^{\circ}$ | 13 ± 1^{bc} | 46 ± 1^{e} | - |
| Cycloh | - | - | _ | - | - | 25 ± 1^{d} |

AMC30: Amoxicillin, TE30: Tetracycline, Cycloh: Cyclohexamide. SA: S. aureus, EC: E. coli, ST: S. typhi, KP: K. pneumonia, SB: S. boydi, CA: C. albicans.

Values are means \pm SD. The outcomes not sharing a common superscript letter in the same column are significantly different at *P*<0.05.

Table 4

Antimicrobial activity of ethyl acetate extract of promising isolates against MRSAs.

| Crude extracts and | Zone of inhibitions (mm) | | | |
|----------------------|--------------------------|-----------------------|--|--|
| standard antibiotics | MRSA2 | MRSA4 | | |
| Ab18 | 20 ± 1^{d} | $15\pm1^{\mathrm{b}}$ | | |
| Ab28 | $0\pm 0^{\mathrm{a}}$ | 0 ± 0^{a} | | |
| Ab43 | $16\pm1^{\rm b}$ | $17\pm1^{\circ}$ | | |
| Ab18&43 | $18\pm1^{\circ}$ | $18\pm1^{\circ}$ | | |
| Penicillin | $0\pm 0^{\mathrm{a}}$ | $0\pm0^{\mathrm{a}}$ | | |
| Methicillin | 0 ± 0^{a} | $0\pm0^{\mathrm{a}}$ | | |
| VA30 | 30 ± 1^{e} | 20 ± 1^{d} | | |

VA30: Vancomycin, MRSAs: Methicillin resistant *S. aureus* strains. Values are means±SD. The outcomes not sharing a common superscript letter in the same column are significantly different at *P*<0.05.

4. Discussion

Antibiotics are the most important bioactive compounds for the treatment of infectious diseases. But now, because of the emergencies of multi-drug resistant pathogens, there are basic challenges for effective treatment of infectious diseases. Thus, due to the burden for high frequency of multidrug resistant pathogens in the world, there has been increasing interest for searching effective antibiotics from soil actinomycetes in diversified ecological niches^[22].

In the present study, the randomly selected soil samples were taken from rhizosphere, industrial and public waste disposal areas for isolation of actinomycetes. The successful isolation of actinomycetes from environmental samples requires an understanding of the potential soil sample areas and environmental factors affecting their growth. Previous studies showed that selection of different potential areas such as rhizosphere soil samples were an important activity for isolation of different types of potent antibiotic producing soil actinomycetes^[23].

The present study of primary screening using single streak

methods indicated that, eight (26.7%) out of 30 actinomycete isolates showed potential antimicrobial activity against one or more test bacteria and/or fungus. This result (26.7%) is higher than 21.88% and less than 59.09% from previous reports^[22,24].

Observation of clear inhibition zones around the wells on the inoculated plates is an indication of antimicrobial activities of antibiotics extracted from actinomycetes against test organisms. Gurung *et al.*^[5] reported 0–18 mm inhibition zone of crude extracts against selected test organisms. From the present study, a range of recorded inhibition zone of crude extract from isolates against test organisms were 0–40 mm (including the combination effect of extracts) which were higher than the result reported by Gurung *et al*^[5].

The previous study indicated that, the inhibition zone of crude extracts from isolates against MRSAs ranged from 0-15 mm^[25]. In this study, inhibition zone of crude extracts from three isolates against MRSAs ranged from 0-20 mm which was found to be good when compared to Yucel and Yemac's results^[25]. The results of the present study were interesting and encouraging because the crude extracts from the isolates may have promising antibiotics for treatment of MRSAs. According to the present result, vancomycin had (20±1) mm and (30±1) mm inhibition zone against MRSA4 and MRSA2 respectively, which had greater inhibition zone when compared to crude extracts from the isolates. But, the crude extracts will show good inhibition zones like vancomycin after purification. Therefore, further purification process is significant to get pure antibiotic substance for the application of treatment of different pathogenic microorganisms.

Karmegan *et al.* reported that the combination of different plant extracts have shown a possibility and good antimicrobial activity against food borne diarreagenic bacteria^[26]. In the present study, the combined effect of the crude extracts of promising isolates [especifically, Ab18 (37 ±1) mm and Ab43 (30±1) mm] have shown significantly ($P \le 0.05$) higher antimicrobial activity in comparison with the individual extract against *S. boydi* ATCC9289 (40±1 mm).

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Actinomycetes are the most widely distributed groups of microorganisms in nature which primarily inhabit the soil. They are the most economically and biotechnologically valuable prokaryotes. They are responsible for the production of about more than half of the discovered bioactive secondary metabolites notably antibiotics, antitumor and immunosuppressive agents, and enzymes.

Research frontiers

Studies have been performed to isolate and screen actinomycetes from different soil samples for their antibiotic production. Isolates from different ecological sites and depths of soil were obtained, and promising actinomycete isolates were selected by primary screening followed by secondary screening using standard bacterial pathogens and clinical isolates of MRSAs and fungi.

Related reports

Actinomycete isolates and their crude extracts' activities against MRSAs ranged from 0-20 mm diameter inhibition zone. This result was higher than that of Yucel and Yemac (2010), which was from 0-15 mm. The variations may be explained by the fact that actinomycetes are very diverse group showing variations in different geographical areas.

Innovations & breakthroughs

Data about antibiotic producing actinomycetes in this particular area is scarce. Thus, this study has screened antibiotic producing actinomycetes from different soil samples which may give an important clue and lead towards finding novel secondary metabolites (antibiotics) which can be up scaled or optimized for treatment of drug-resistant pathogens.

Applications

The results of the present study may be significant to initiate many researchers in this particular area to look for different sources of samples for searching new bioactive secondary metabolites like antibiotics.

Peer review

This is a good study in which the authors searched different soil samples at different ecological areas of Gondar. There is no report about this industrially and biotechnologically important actinomycetes for the production of secondary metabolites like antibiotics in this area before.

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