Assessment of Antimicrobial Activity of Actinobacteria Inhabiting Harsh Conditions

Samar M. El-Shahidy [1], Dewedar, A. [1], Ishrak Khafagi [1], and Samira R. Mansour [1]

[1]*Department of Botany, Faculty of Science, Suez Canal University, Ismailia, Egypt.*

Abstract:

In this study, 63 isolates of actinobacteria were isolated from marine sediment and sandy soil of Saint Katherine, Egypt and examined for antimicrobial activity. Both sites were highly inhabited by rare promising Actinobacteria species. Recovered Actinobacteria belonging to genus *Streptomyces, Actinomadura, Micromonospora, Microtetraspora, Nocardia, Pseudonocardia Nocardiopsis, Actinoplanes, Pilimelia and Spirellospora.* Their extracts exhibited wide antimicrobial activities towards 4 clinical pathogens. Out of them 49.21% showed potent inhibitory effect against test pathogens in primary and secondary screening methods in which genus *Actinomadura* was significantly the highest followed by genus *Streptomyces* then genus *Micromonospora*. The crude extracts showed 0.3-20 mm inhibition zone in disc diffusion method against tested pathogens. The active compounds were purified by preparative TLC, which showed retention factor value (RF) of 0.14-0.857 with isolated bioactive Actinobacteria and identified using different reference antibiotics. In bioautography, TLC spot of *Actinomadura roseoviolacea* with RF of 0.26 showed maximum activity with 40 mm inhibition zone and nearby Tetracycline reference antibiotic. This isolate show safe hemolytic activity *In vitro* analysis.

Keywords: Actinobacteria, Antimicrobial extraction, marine habitat, Saint Katherine area, hemolytic activity, TLC, and Bioautography

Introduction

With the drug resistance of pathogenic microorganisms increasing at alarming rate, there is an increase in the demand for newer and safer antibiotics with lesser side effects ***(Gupte et al, 2002).*** In future, the best alternate to meet the increasing demand of safe and cost effective drugs is natural products from actinobacteria ***(Behal, 2003).*** Actinobacteria are a well-known source of various secondary metabolites such as antibiotics, enzymes, pesticides, herbicides, immunomodulators, anti-infective agents and anticancer agents ***(Takahashi and Omura, 2003; Newman and Craig, 2007; Chaudhary et al, 2013).*** Majority

of antibiotics are derived from genus Streptomyces ***(Watve et al, 2001).***

Antibiotics of actinobacterial origin have evidence a wide variety of chemical structures including aminoglycosides, anthracyclines, glycopeptides, β-lactams, nucleosides, peptides, polyenes, polyketides, actinomycins and tetracyclines ***(Waksman, 1968 and Basavaraj et al, 2010).*** Many of actinobacterial antibiotics developed into drugs at industrial level and used for treatment of wide range of infectious diseases in human, veterinary and agriculture sectors ***(Kelecom, 2002).*** An ideal antimicrobial agent is that exhibit selective toxicity, which means the drug, is harmful to a pathogen without being harmful to the host. Often, selective toxicity is relative rather than absolute, this implies that a drug in a concentration tolerated by the host may damage an infecting microorganisms. Selective toxicity may be a function of specific receptors required for drug attachment or it may depends on the inhibition of biochemical events essential to pathogen but not to the host ***(Jawetz and Adelbergs, 2007).***

The inconvenient effects that follow the administration of antibiotics are numerous and varied and may involve nearly every organ system. Disturbances of the urinary tract and renal damage are the most important undesirable effect of antibiotic. Hemolytic anemia and other disorders of hematopoietic system also are one of the most important bad effects of antibiotics ***(Shattil et al, 1980)***. The hemolytic episode occurs abruptly usually in the first week of therapy. Nausea, fever, jaundice and pallor are consequence of hemolytic anemia. Marked decrease in erythrocytic count and hemoglobin concentration are the common laboratory findings of hemolytic anemia ***(Levine et al, 1973).***

Due to large geographic variations in Egyptian soil type, it is quite likely that the distribution of antibiotic producing actinobacteria is also diverse. Therefore, searching for new screened antibiotic producing isolates and the verification of their undesirable effect, as hemolytic activity to human erythrocytes *In vitro*, were explored in this study.

Matrials and methods

Isolation and characterization of Actinobacteria.

Sixty three actinobacterial isolates were assessed for their antimicrobial and hemolytic activity. Actinobacterial genera were isolated on starch casein agar from marine sediment of El-Shat' beach, Ismailia (three isolates) and arid sandy soil of Ferran valley, Saint Catherine (Sixty isolates). Isolated Actinobacteria were identified microscopically and biochemically and screened for their antimicrobial activities. The isolates screened were belonging to

Streptomyces spp. (Total 10, one isolate from marine soil and 9 isolates from harsh sandy soil), *Actinomadura spp.* (26 isolates from harsh sandy soil), *Micromonospora spp.* (7 isolates from harsh sandy soil), *Microtetraspora spp*. (1 isolate from harsh sandy soil), *Nocardiaspp*. (total 5 isolates from harsh sandy soil), *Psudonocardia spp*. (total 5 isolates,one isolate from marine soil and four from harsh sandy soil), *Nocardiopsis spp.* (5 isolates, one from marine soil and four from harsh sandy soil), *Actinoplanes sp*. (2 isolates from harsh sandy soil), *Pilimelia columellifera* (1 isolate from harsh sandy soil), and *Spirellospora spp.* (1 isolate from harsh sandy soil).

Test pathogenic microorganisms

The following bacterial strains: uropathogenic NRRL B-3704 *Escherichia coli*, NRRL B-767 *Staphylococcus aereus*, NRRL B-23 *Pseudomonas aeruginosa* and y-12983 *Candida albicans* were used in this study. These tested organisms were gently provided by the United States Department, Agricultural Research Services (USDA), USA.

Primary screening of antimicrobial activity

Ten days incubation actinobacterial agar culture was tested by disc overlay bioassay on pathogens seeded agar plates following the method of ***Teresa et al (1991)*** and ***Sharma et al (2011).*** Agar plates were incubated at 37°C and 25°C for bacteria and yeast respectively for 48 hours during which activity was evidenced by the presence of a zone of inhibition surrounding the disc. The antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm)

Preparation of Culture Filtrates

Production of antimicrobial metabolites was carried out by shake flask fermentation method according to the method described by ***Anindita et al (2008)***. Fifty milliliters of the production media in 250 ml Erlenmeyer flask was inoculated with the actinobacterial isolates and incubated in a rotary shaker (240 rpm) at 30° C for 7 days. Actinobacterial culture was centrifuged at 1600 Xg for 20 minutes. Supernatant was evaluated as culture filtrate for antimicrobial activity prior to extraction method.

Extraction of antimicrobial metabolites from culture filtrates

Another set of cell sediment and supernatant, after centrifugation, were used for extraction and purification of antimicrobial metabolites by solvent extraction technique following the process described by ***Westly et al (1979)*** and ***Alimuddin et al (2011).*** Each actinobacterial pellet and supernatant are mixed separately with ethyl acetate and choloroform , in the ratio of 1:1 (v/v) and shaken vigorously for 1 hour for complete extraction. The aqueous layer and organic layer of both cell sediment and supernatant in both solvent were used to determine the

antimicrobial activity. Both culture filtrate and extracted metabolites were tested by disc diffusion and agar gel diffusion methods.

Secondary screening for antimicrobial activity

Two different techniques were performed to assess the antimicrobial activity in actinobacterial broth culture. **Agar gel diffusion method,** in which specific amount of actinobacterial culture filtrate (100 µL) were tested in specific well bored on pathogens agar plates ***(Jeffrey, 2008).*** Disc diffusion method was performed using overnight saturated discs of Watt man filter paper No.3 with extracted antibiotics and wrapped up the surface of seeded plates. Plates were incubated for 48 hour. The zone of inhibition was measured and recorded.

Identification of antimicrobial compounds

a. Thin layer chromatography

Antimicrobial extracts of actinobacterial isolates were identified using Liquid-liquid fractionation manner following the procedures of ***Pandy et al (2004)***. Silica gel plates (sorbent), 20×20 cm, 1mm thick with ascending development were prepared and activated at 150°C for half an hour. Ten microliters of the ethyl acetate fractions and reference antibiotics (Tetracycline, Oxytetracycline, Streptomycin, Neomycin, Ampicillin and Chloramphenicol) were applied on the plates and the chromatogram was developed using chloroform : methanol (4:1 v/v) as solvent gradient system. The plates were run twice at the same condition in duplicate set. Spots of chromatogram were visualized in UV chamber with short wavelength (254 nm).

b.Bio autography.

Chromatogram spots were cut/scratched and applied on agar plates seeded with tested organisms and incubated overnight at 37°C in sterile condition according to the method of ***Irena and Grzelak (2010).*** Inhibition zones were noted and the RF values of the antimicrobials were determined according the equation

$$RF = \frac{\text{Distance run by sorbent}}{\text{Distance run by solvent.}}$$

Hemolytic activity of extracted antibiotics

The hemolytic activity of potent antimicrobial producing actinobacteria was measured following the protocol of ***Malagoli (2007)*** in vitro conditions. In this assay two fold dilution of actinobacterial metabolites were prepared and mixed with 0.5 ml of 10% washed RBCs. The mixture was incubated for 30 minutes at 37°C and then centrifuged at 1500 rpm for 10 minutes. The free haemoglobin in supernatant was measured using UV-Vis spectrophotometer at 540 nm. Distilled water and triton was used as negative and positive hemolytic controls.

Results

Primary and Secondaryscreening

Among the 63 isolates screened for antibacterial activity, 31 actinobacterial isolates showed significant antimicrobial activity with a total percentage 49.21% (Table 1). Bioactive genera belonged to genus *Actinomadura* was significantly the highest followed by genus *Streptomyces* then genus *Micromonospora* (Fig.1). However, the genera: *Nocardia, Pseudonocardia*, and *Nocardiopsis* were equally in number of the active isolates. Meanwhile, *Actinomadura* isolates showed the most brood spectrum activity against all tested organisms (Fig. 1). Sixteen actinobacterial isolates had a broad spectrum antimicrobial activity against more than 2 challenged pathogenic strains (Table 1)

Bacterial and fungal antagonistic isolates, detected by agar overlay or agar gel diffusion and disc diffusion technique, showed variation of inhibition zone (Table 1). All positive antimicrobial isolates (31) have inhibitory effect against Gram +ve *Staphylococcus aureus*(Fig. 1 and 2). However, only 14 isolates had inhibitory effect against Gram – ve *Escherichia. Coli* and recorded a percentage of 22.22% of total screened isolates (Table 2). Meanwhile, 17 isolates were effective against *Pseudomonas aeruginosa* and 15 isolates were effective against *Candida albicans* strain (Fig. 1) and represented by percentage of 26.98%, 23.81% respectively.

Extraction of active metabolites using different solvents showed that ethyl acetate solvent was better than chloroform as solvent phase. It also recorded that ethyl acetate extract was not significantly different than activities carried out by crude broth without any extraction (cell-free broth media, primary screening). Meanwhile, tested aqueous extracted phase was less active than solvent phase. Secondary screening by gel and disc diffusion method of culture filtrate and culture extracts showed low activity when compared to primary screening (Fig. 1). This may result from agar rigidity which delay the distribution of antimicrobial substances and interrupt their activity.

Thin Layer Chromatography

With respect to identification of extracted antibiotics, from selected actinobacterial isolates with high antimicrobial activity using thin layer chromatography (TLC) was listed in Table 2. RFvalue of the tested compound of isolates were ranged from 0.14 to 0.857 similar to RF of reference antibiotics (Table 2). It was clearly found that, the extracted antibiotics from *Actinomadura sp*. may be close to the standard Tetracycline, since they have close RF value. Therefore, close RF value for standard antibiotic used in this study for comparison can give us an idea for the proposed antibiotic

compounds that can be produced by tested isolates (Table 2).

Bioautography

In Bioautography, *Actinomadura roseoviolacea* (Isolate 9), *Nocardiopsis sp.* (isolate 45) and Streptomyces sp.(isolate 7) gave mean diameter value of inhibition zone, of the tested pathogens, of 40, 36, 12 mm respectively. Meanwhile, the reference antibiotic Tetracycline, Oxytetracycline and Chloramphenicol showed close inhibition zone diameters, in respective way, as follow: 40, 50 and 56 mm diameter respectively. These data may confirm the proposed expectation for identification of unknown antibiotic compounds which also had the close results of RF values.

Hemolytic activity

Concerning to hemolysis of human blood group O using the tested isolates, data showed different degrees of hemolysis and some of them had very slight effect which considered as negative results (Fig. 3). Hemolytic activity of extracellular antimicrobial compounds, for the tested actinobacterial isolates, showed that most of *Actinomadura sp*. had no hemolytic effect. Meanwhile, complete hemolysis of human blood group O was seen by *Nocardiopsis sp*. (Isolate 27). Partial hemolysis was also seen with other genera like *Micromonospora echinospora echinospor*a (Iso. 51), *Nocardiopsis sp.* (Is.47).

Table 1: Antimicrobial activity and diameter of inhibition zones (mm) of actinobacterial genera isolated from studied sites.

Isolae code.	Identified genera	*NRRL (B767) S. aureus*			*NRRL(B34) E. coli*			*NRRL (B-23) P.aerugi nosa*			*NRRL (Y-12983) C. albicans*	
		Activ ity	(ø)		Activity	(ø)		Acti vity	(ø)		Activity	(ø m m)
1	*Actinomadura roseoviolacea*	+++	14		+++	20		+++	12		++	8
2	*Streptomyces sp.*	++++	16		-	-		++	8		-	-
3	*Actinomadura sp.*	+++	14		++++	16		+++	12		++	6
4	*Streptomyces sp.*	++++	16		-	-		-	-		-	-
7	*Streptomyces sp.*	++++	18		-	-		-	-		-	-
8	*Streptomyces sp.*	-	-		-	-		-	-		-	-
9	*Actinomadura roseoviolacea*	+++ ++	28		++++	18		++ ++	16		++	10
10	*Pseudonocardia sp.*	-	-		-	-		-	-			-
11	*Streptomyces sp.*	++++	16		-	-		-	-		-	-
12	*Actinomadura yumansis*	+++	14		-	-		+++	12		-	-
13	*Actinomadura sp.*	+++	14		++++	16		+++	12		+	4
14	*Actinomadura verrucocsospora*	++++	16		-	-		-	-		-	-
15	*Actinomadura sp.*	-	-		-	-		-	-		-	-

	Subsp of .43											
16	***Nocardia orientalis***	++	**10**		-	-		-	-		-	-
20	***Actinomadura roseoviolacea***	++	**10**		+++	**22**		++	**10**		++	**8**
21	***Streptomyces***	-	-		-	-		-	-		-	-
22	***Streptomyces***	-	-		-	-		-	-		-	-
23	***Actinomadura roseoviolacea***	+++ ++	**22**		++++	**18**		+++	**12**		++	**8**
24	***Actinomadura livida***	-	-		-	-		-	-		-	-
25	***Streptomyces***	-	-		-	-		-	-		-	-
26	***Streptomyces***	-	-		-	-		-	-		-	-
27	***Nocardiopsis***	-	-		-	-		-	-		-	-
28	***Actinomadura roseoviolacea***	+++	**14**		+++	**20**		++	**10**		++	**8**
29	***Actinomadura vinacea***	+++ ++	**26**		++++	**16**		++	**10**		++	**8**
30	***Actinomadura roseoviolacea***	+++	**20**		+++	**20**		++	**10**		++	**8**
31	***Pseudonocardia*** *spinosa*	+++	**14**		-	-		-	-		-	-
32	***Micromonospora echinospora ferruginea***	-	-		-	-		-	-		-	-
33	***Actinomadura***	-	-		-	-		-	-		-	-
34	***Micromonospora carbonacea aurantica***	-	-		-	-		-	-		-	-
35	***Micromonospora carbonacea carbonacea***	-	-		-	-		-	-		-	-
37	***Pseudonocardia***	++	**10**		++	**10**		-	-		+++	**14**
38	***Nocardiopsis dassonovili***	++++	**16**		-	-		++ ++	**16**		-	-
39	***Actinomadura sp.***	-	-		-	-		-	-		-	-
39'	***Nocardia medettranie***	-	-		-	-		-	-		-	-
40'	***Nocardiopsis***	-	-		-	-		-	-		-	-
40	***Nocardia medettranie***	-	-		-	-		-	-		-	-
41'	***Pseudonocardia***	-	-		-	-		-	-		-	-
42	***Actinomadura***	++	**10**		-	-		-	-		++	**7**
42'	***Pseudonocardia***	-	-		-	-		-	-		-	-
43	***Actinomadura***	++	**10**		-	-		-	-		++	**6**
44	***Micromonospora echinospora ferruginea***	-	-		-	-		-	-		-	-

Ø mm; diameter of inhibition zone

Activity: +; 0-5 ø mm, ++; 5-10 ø mm, +++; 10-15 ø mm, ++++; 15-20 ø mm, +++++; > 20 ø mm

Antimicrobial activity and diameter of inhibition zones (mm) of actinobacterial genera isolated from studied sites. (Continued).

Isolate Code	Identified genera	NRRL (B-767) S. aureus			NRRL (B-3704) E. coli			NRRL (B-23) P. eruginosa			NRRL (Y-12983) C. albicans	
		Activity	(ø mm)		Activity	(ø mm)		Activity	(ø mm)		Activity	(ø mm)
45	*Nocardiopsis*	++++	20		-	-		-	-		-	-
46	*Streptomyces*	++++	20		-	-		+++	12		-	-
47	*Nocardiopsis flava*	++++	18		++	8		-	-		-	-
48	*Actinomadura livida*	-	-		-	-		-	-		-	-
51	*Micromonospora echinospora echinospora*	+++	14		-	-		++	6		-	-
52	*Micromonospora carbonacea aurantica*	-	-		-	-		-	-		-	-
53	*Spirellospora*	-	-		-	-		-	-		-	-
54	*Actinoplanes*	-	-		-	-		-	-		-	-
55	*Actinoplanes italicus*	+++ ++	22		-	-		++	10		-	-
56	*Actinomadura cremea*	++	6		-	-		-	-		-	-
57	*Pilimelia collumillefera*	-	-		-	-		-	-		-	-
58	*Actinomadura roseoviolacea*	+++	*23*		+++	*14*		++	*16*		++	*9*
59	*Actinomadura helevata*	++	*10*		+	*2*		-	-		++	*10*
60	*Actinomadura*	-	-		-	-		-	-		-	-
61	*Microtetraspora neveoalba*	-	-		-	-		-	-		-	-
62	*Nocardia otitidiscaviarum*	-	-		-	-		-	-		-	-
63	*Nocardia otitidiscaviarum*	-	-		-	-		-	-		-	-
64	*Actinomadura*	-	-		-	-		-	-		-	-
65	*Actinomadura spiralis*	-	-		-	-		-	-		-	-
67	*Actinomadura vinacea*	++	*10*		++	*6*		+++	*20*		+	*5*
68	*Actinomadura livida*	+++	*12*		-	-		-	-		-	-
69	*Micromonospora carbonacea carbonacea*	-	-		-	-		-	-		-	-

Ø mm; diameter of inhibition zone

Activity: +; 0-5 ø mm, ++; 5-10 ø mm, +++; 10-15 ø mm, ++++; 15-20 ø mm, +++++; > 20 ø mm

Table. 2: Proposed identification of active compounds extracted by ethyl acetate and separated by TLC in comparable to standard antibiotics

Isolate No.	Actinomycete species	R_F †	Proposed Reference antibiotic	
			(500 mg/ml diluent)	R_F ††
9	*Actinomadura roseoviolacea*	0.26	Tetracycline	0.25
37	*Pseudonocardia sp.*	0.21	Tetracycline	0.25
43	*Actinomadura sp.*	0.15	Oxytetracycline	0.14
13	*Actinomadura sp.*	0.15	Oxytetracycline	0.14
23	*Actinomadura roseoviolacea*	0.25	Tetracycline	0.25
59	*Actinomadura helevata*	0.16	Oxytetracycline	0.14
7	*Streptomyces*	0.42	Chloramphenicol	0.35
45	*Nocardiopsis sp.*	0.14	Oxytetracycline	0.14

†, RF value (Retention factor) of organic phase tested compounds,
†† Rf value of standared antibiotics
TLC condirions, *Sample*: Ethyl acetate fraction; *Sorbent gel*: Silica; Plate size :20X20 cm;
***Mobile phase*: chloroform: methanol(4:1v/v); Development: ascending;**
Running: twice at same condition;
drying at room temperature; Detection :UV 254 nm short wavelength

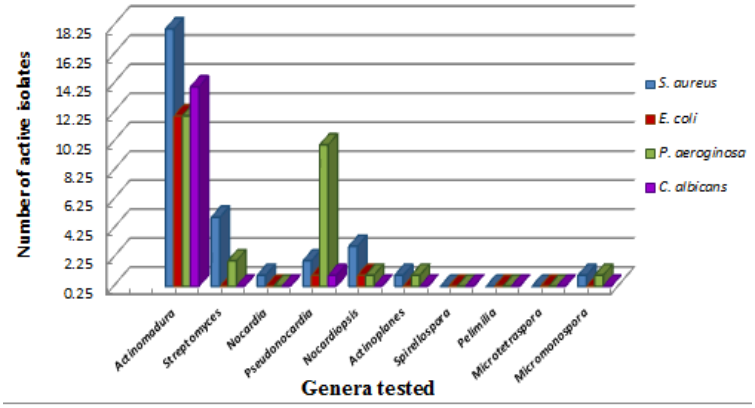

Fig. 1: Number of active genera against Gram positive bacteria, *Staphylococcus aureus*; Gram negative bacteria, *E.coli* and *Pseudomonas aeroginosa*; and *Candia albicans*.

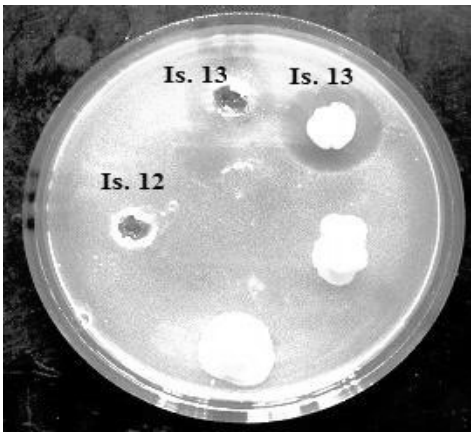

Fig. 2: Inhibitory effect of *Actinomadura sp*. (isolate 13) detected by agar disc overlay bioassay and gel diffusion method.

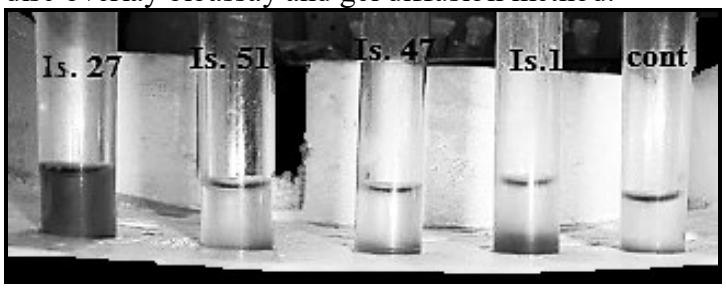

Fig.3: Hemolytic activity of extracellular antimicrobial compounds of selected actinobacterial isolates.

Discussion

This study shows that the test actinobacterial isolates have the potential to act as sources of antibacterial agents against human pathogens and put more emphases on actinobacteria as a known source of antimicrobial agents. In this study, 31 out of 63 showed antimicrobial activities. All active isolates (31) were able to inhibit Gram positive bacteria (NRRL B-767 *Staphylococcus aureus*). These isolates were belonging to 18 *Actinomadura* species, 5 *Streptomyes* sp., one *Nocardia sp*., 2 *Psuedonocardia,* 3 *Nocardiopsis*, one *Actinoplanes* and one isolate of *Micromonospora sp*. However, against Gram negative pathogenic organisms (NRRL B- 3704 *Escherichia coli*) only 14 isolates showed activity; most of them were belonging to genus *Actinomadura*. For another G –ve pathogens (NRRL B-23 *Pseudomonas aeruginosa*), 17 isolates were active. For fungal pathogens (NRRL Y- 12983 *Candida albicans*), 15 isolates were active from which 14 isolates were belonged to genus Actinomadura. These data were different than usual studies where genus Streptomyces is always the high producing organisms ***(Kumar et al, 2012).***

The antimicrobial activity data recorded by *Nocardiopsis*, *Micromonospora*, *Actinoplanes* and *Nocardia* are in agreement with data obtained *by* ***Harald et al (2007)***. In their study, they proved that that *Micromonospora*, *Actinomadura ,Nocardiopsis, Streptomyces*, and *Streptosporangium* genera were the most common antibiotic producers from actinomycetes. The source of their isolates was from shallow water sediments associated with successful amplification of bioactive polyketide genes in genome of these strains.

The active isolates of primary screening when subjected to secondary screening, showed different activities. Some of active isolates lost their capability for pathogenic inhibition, while some other showed moderate activities. In contrary, some other improved their activities. According to ***Bushell (1993)***, during the screening of the novel secondary metabolite, actinomycetes isolates are often encountered to show antibiotic activity on agar but not in liquid culture. This may explain the losing activity of some of isolates.

Ethyl acetate extraction of extracellular and intracellular actinobacterial isolates produced activity when tested against some pathogens, especially with organic phase. Crushed cell fraction and solvent fraction showed potent antibacterial and antifungal activities against tested microbes. *Actinomadura* and *Streptomyces* species recorded the largest antibacterial spectrum with tested pathogens. These results are in confirmation with the obtained data recorded by ***Mincer et al (2002)*** and ***Parente and Riccardi (1998)***. In their studies, they declared that ethyl extract of actinobacterial isolates show evidence of antimicrobial activity against Gram-positive species such as *Clostridium perfringens*, *E. faecalis*, *S. aureus* and different *Bacillus sp*.

To have partial characterization of obtained antibiotic, TLC separation carried out. Our results presented proved that extracted compounds may be similar to the standard antibacterial compounds since they were visible on bioautogram with similar RF values. In addition, their inhibition zones were associated with yellowish green spots which had been detected under UV radiation. This observation may reflect the close relation with the standard antibiotics used. For proper identification of the antimicrobial extracts, further studies should be carried out.

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الملخص العربى

قياس النشاط الضد ميكروبى لبكتيريا الاكتينو التى تسكن البيئات القاسية

فى هذه الدراسة تم عزل ٦٣ معزولة من بكتيريا الاكتينو من الرواسب البحرية بالاسماعيلية والتربة الرملية من سانت كاترين بمصر وتم اختبار نشاطها كمضاد حيوي ضد بعض الميكروبات المرضية . اثبتت هذه الدراسة ان هذه الاماكن غنية بانواع مختلفة من بكتيريا الاكتينو النادره تحت جنس ستبرتوميسيس ، اكتينومديورا، ميكرومونوسبورا ، ميكروتتراسبورا، نوكارديا، سيدونوكارديا، نوكارديوبسيس، اكتينوبلانس، بيليميليا ، سبيرلوسبورا.
قد اظهرت خلاصة هذه الانواع من بكتريا الاكتينو نشاط ضد ميكروبى واسع ضد ٤ انواع من الميكروبات البكتيرية المرضية الاكلينيكية . ٤٩,٢١% من انواع بكتيريا الاكتينو لها نشاط مثبط قوى لهذه الميكروبات المرضية وذلك عندما تم الكشف عنها بالطرق الاولية والثانوية للكشف الضد ميكروبى والتى اثبتت ان جنس الاكتينو مديورا قد اعطى نتائج اعلى من جنس ستربتوميسيس وجنس ميكرومونوسبوروا . قد اظهرت خلاصة الميكروبات النقية انها تثبط الميكروبات المرضية فى مساحة واسعة يصل قطرها ٠,٣-٢٠ مل وذلك باستخدام طريقة انتشار القرص . وقد تم تنقية المواد المستخلصة من بكتريا الاكتينو بواسطة جهاز الكروماتوجرافى رقيق الطبقة والذى اثبت ان قيمة معامل الاحتجاز فى المواد المستخلصة من ٠,١٤-٠,٨٥٧ فى المواد النشطة من بكتريا الاكتينو والتى تم التعرف عليها باستخدام بعض المضادات الحيوية المرجعية وقد اثبتت الاكتينومديورا اعلى معامل احتجاز يصل الى ٠,٢٦ مع منطقة منع قطرها ٤٠ مل مع المضاد الحيوى تتراسيكلين المرجعى وقد اثبت هذا النوع من بكتريا الاكتينو انه ليس له نشاط تكسيرى على كرات الدم الحمراء عند فحصها فى المعمل.