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Taificidin1 and Taificidin2, Two Anti-microbial Agents Isolated from the Fermentation Broth of *Streptomyces roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA17

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ABSTRACT

Isolation of antimicrobial agents from actinomycetes isolated from EL-Taif area, Kingdom of Saudi Arabia, two streptomyces isolates showed remarkable antimicrobial activity against tested organisms. The two isolates were previously identified according to the recommended keys as *Streptomyces roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA17. The active metabolites of *St. roseodistaticus* TA15 and *St. lavedofoliae* TA17 were extracted by using different organic solvents, purified using both Thin Layer Chromatography (TLC) and column chromatography. The active compounds were concentrated and investigated for their physical characteristics and elemental, spectroscopic and biochemical analysis and their Minimum Inhibition Concentration (MIC) were determined. The cytopathic effect of the two antimicrobial agents on the vero cell virus, the neutralization test in monolayer cell cultures for antiviral test on coxasackie B4, hepatitis A and herpes simplex (cox B4, HAV and HSVI) viruses was also determined. Aim of this study is to identify the antimicrobial activity of actinomycetes extracts isolated from Saudi Arabia. We suggested the name "Taificidin 1" and "Taificidin 2" for the antimicrobial agent which extracted from *St. roseodistaticus* TA15 and from *St. lavedofoliae* TA17 as two active compounds isolated for first time from EL-Taif region, KSA.

Key words: Actinomycetes, *S. roseodistaticus*, *S. lavendofoliae*, antimicrobial agents, coxasackie B4

INTRODUCTION

Actinomycetes is one of the most attractive families of industrial bacteria on account of their superior potential for producing valuable secondary metabolites including antibiotics, anti-cancer drugs, immunosuppressors and enzyme inhibitors (Zitouni *et al*., 2004a). The species belonging to the genus Streptomyces constitute 50% of the total population of soil actinomycetes and 75-80% of the commercially and medicinally useful antibiotics have been derived from this genus (Lazzarini *et al*., 2000). The list of novel microorganisms and products derived from poorly explored areas of the world like China, Australia, Antarctica and Jordan suggests that a careful exploration of new habitats might continue to be useful (Zitouni *et al*., 2004a). The search for new antibiotics continues to be almost importance in research programs around the world because the increase of the resistant pathogens and toxicity of some used antibiotics. Among microorganisms actinomycetes are one of the most investigated groups particularly members of the genus Streptomyces from which, a large number of antibiotics was obtained and studied (Gupte *et al.,* 2000). The vast majority of actinomycetes have originated from soil (Shearer, 1997) and their isolation method deal almost exclusively with those suitable for *Streptomyces* species which grow rapidly on soil dilution

plates. However, in recent years, the rate of discovery of new antibiotics in the genus Streptomyces was declining and isolation of other actinomycete genera, appeared to be necessary to assess the health hazard and to and novel strains producing commercially valuable antibiotics. With the discovery of new antibiotics from strains of Actinomadura, Micromonospora, Saccharothrix and Streptosporangium, increased emphasis was placed on developing methods for the isolation and identification of non-streptomycete actinomycetes (Shearer, 1997). During a screening program for Streptosporangium, strains can produce valuable substances of biotechnological interest search of potent antimicrobial products, was focused on antibiotic producing rare actiomycetes. Selective methods were used to isolate new strains producing new antibiotics (Zitouni *et al.*, 2004b). It has been also found that Streptosporangium strains can produce valuable substances of biotechnological interest (Ammor *et al.*, 2008). These promising results emphasize the need to continue the researches in this way. My objective of my research is to identify the characteristics of antimicrobial activity of actinomycets extracts to be used as antimicrobial agents or anticarcinogenic candidates.

MATERIALS AND METHODS

Screening of antimicrobial agents from actinomycete isolates previously isolated at 2005-2006 (15 and 17): This study was started since 2005 at kingdom of Saudi Arabia, Taif region for isolation of new strains of actinomycets. For the investigation of the antagonistic properties of the microbial isolates, the classical diffusion method for testing the antimicrobial activities were used for such a purpose (agar well method). The following test microorganisms were used for such a purpose:

Bacteria (ProKaryotes):

- Gram-positive cocci: *Micrococcus lutues*, ATCC 9341
- Gram-positive bacilli: *Bacillus subtilis*, NCTC 10400
- Gram-negative bacteria: *Escherichia coli*, NCTC 10416 and *P. aeruginosa*, ATCC 10145

Fungi (EuKaryotes):

- Unicellular fungi: *Candida albicans*, IMRU 3669
- Filamentous fungi: *Aspergillus flavus*, IMI 111023 and *Penicillium chresogenum*

Fermentation, extraction, separation and purification of the antimicrobial agent(s) produced by most potent actinomycete isolates (15 and 17)

Fermentation: A 50 mL sample of seed medium in a 250 mL flask was inoculated with a mycelia suspension of actinomycete strains (15 and 17) and incubated at 30°C and 250 rpm on a rotary shaker. After 3 day this first-stage was transferred to 500 mL seed medium in a 2 L conical flask and incubated under the same conditions for another 3 day. This second-stage was used as the inoculum for fermentation in 5 L fermentor. The pH was adjusted at pH 7. The temperature was adjusted at 30°C, the agitation at 250 rpm and aeration rate at 1 vvm. Foam was suppressed by sterile sunflower oil (El-Tayeb *et al.*, 2004).

Extraction: The clear filtrate was collected then the extraction process was carried out. The efficiency of the extraction was investigated by using five organic solvents namely, chloroform,

hexan, ethyl acetate, n-Butanol, petroleum ether. Equal volumes of the broth was mixed with the organic solvent (1:1 v/v) and shacked vigorously in a separating funnel. The mixture was left for few minutes, after which the organic layer was separated and collected for further studies. The organic phase was collected, evaporated under reduced pressure by using a rotary evaporator. The evaporation was continued until viscous syrup was obtained. The residual Syrup was dissolved in the least amount of solvent and filtered through whatman No. 1 filter paper.

Purification

By thin layer chromatography (TLC): Purification of the antimicrobial agent(s) into its individual components has been tried by TLC technique using a solvent system composed of Chloroform and Methanol (24:1 v/v). It was carried out by spotting 10 micron on silica gel plates which were developed for 2 h. Detection of the purification of the antimicrobial agent(s) was accomplished by a microbiological procedure. This was carried out by developing different spots on thin layer plates covered with agar plates seeded with bacteria (Gram +ve and Gram –ve) and fungi (unicellular and filamentous fungi) and then incubating the agar plates for 24 h for bacteria at 37°C, whereas yeast incubating the agar plate for 48 h at 30°C and 72 h for fungi at 30°C. The antimicrobial components are visualized as zones of inhibition on the agar plates.

By column chromatography: A column of 2×70 cm diameter was used for this purpose. The column was packed with silica gel (Prolabo). Since chloroform and methanol (9:1 v/v) were used as eluting solvents. One milliliter crude antimicrobial agent extract was added onto top of the silica. The elution mixture of chloroform and methanol (9:1 v/v) was added and then the column was connected to the reservoir. Fifty fractions were collected (each of 5 mL). The antimicrobial activities were performed for each separate fraction.

Characterization and biological activities of the antimicrobial agent(s) biosynthesized by actinomycete isolates (15 and 17): Twenty microliters of the crude preparation of the antimicrobial agent(s) were loaded on paper chromatographic strips. Migration of the antimicrobial agent(s) using separately different solvent systems was calculated as suggested by Bilinov and Khoklov (1970). After ascending, the strips were air-dried and bioautographed according to the method described by Weinstein and Wagman (1978) using *Micrococcus luteus*, ATCC 9341; *Escherichia coli*, NCTC 10416; *Ps. aeruginosa*, ATCC 10145; *Candida albicans*, IMRU 3669 and *Asp. flavus*, IMI 111023 as test organisms. One definite inhibition zone was always recorded.

Determination of the physical and chemical characteristics of the two antimicrobial agent(s)

- **Elemental analysis C, H, O, N, S:** The elemental analysis of was carried out by the National Research Center, Egypt
- **Spectroscopic analysis:** The IR and UV and Mass-spectrum were determined at the National Research Center, Egypt

Reaction of the antimicrobial agent(s) with certain chemical test: This was carried out as follows: Molish's reaction, Sakaguchi reaction, Ninhydrin test, Elrlish reaction, Nitroprusside reaction, Ferric chloride reaction, Fehling reaction, Meyer reaction.

Determination of the Minimum Inhibitory Concentration (MIC) of two antimicrobial agent(s): MIC has been determined by diffusion method, according to Kavanagh (1972).

RESULTS

Screening of antimicrobial activities producing by actinomycete strains: No. 15 and 17: Simple agar well test was used for detecting the activity of crude extracts from isolated strains indicating that actinomycetes strains, No. 15 and 17, secreted an active metabolites which inhibited the growth of all test organisms. These data indicated the broad spectrum activity of the produced metabolites excreted from these two strains against gram (+) and gram (-) strains as well as different fungi strains. Numbers in table indicated the diameter length of clear zone around the well in the plate. Length diameter more than 10 mm showing high activity against microorganisms tested. These results were represented in Table 1. Data of antiviral studies (virucidal activity) which was carried out according to the methods described by Edwin (1969) and Ronald (1996) revealed that, the antibiotics (15 and 17) have no effect on Coxsackie B4, Hepatitis A and Herpes simplex (Cox B).

Fermentation, extraction, separation and purification of the antimicrobial agent(s) produced by most potent actinomycete isolate (15 and 17)

By thin layer chromatography: Purification of the antimicrobial agent(s) into individual components has been carried out by (TLC using a solvent system composed of chloroform and methanol of the ratio (24: 1 v/v). The TLC under UV showed band at $R_f = 0.55$ and band at $R_f = 0.8$ for the compound produced by actinomycete isolate 15 and isolate No. 17, respectively.

By column chromatography: The purification method was carried out using column chromatography. A mixture of chloroform and methanol (9:1 v/v) was used as an eluting solvent. The crude extract was adsorbed on the top of the silica column. Mixture of chloroform and methanol (9:1 v/v) was added by using a pipette. Fifty fractions were collected (each of 5 mL) and tested for antimicrobial activity using an diffusion method against *Micrococcus luteus*, ATCC 9341; *Escherichia coli*, NCTC 10416; *Ps. aeruginosa*, ATCC 10145; *Candida* albicans IMRU 3669 and *Asp. flavus*, IMI 111023 as test organisms. The obtained results in Table 2 revealed that the most active fractions against the tested organisms ranged between 16 to 24 for actinomycete isolate No. 15. These fractions represent the most active compounds isolated for further analysis. It is clear that fraction No. 20 is the best fraction for testing that represents 20, 24, 30, 21 and 19 for the microorganisms *Micrococcus luteu* ATCC 9341, *Escherichia coli* NCTC 10416, *Ps. aeruginosa*, ATCC 10145, *Candida albicans* IMRU 3669 and *Asp. flavus* IMI 111023, respectively.

Table 1: Antimicrobial potentialities of the antibiotic-producing by actinomycete isolates (15 and 17)

	Bacteria					Fungi		
Actinomycete isolates No.	*Micrococcus lutues,* ATCC 9341	*Bacillus subtilis,* NCTC 1040	*Sarcina maxima*	*Escherichia coli ,* NCTC 10416	*Ps. aeruginosa,* ATCC 10145	*Candida albicans,* IMRU 3669	*Asp. flavus,* IMI 111023	*Penicillium chrysogenum*
15	22.0	18.0	25.0	26.0	32.0	24.0	22.0	21.0
17	23.0	20.0	24.0	21.0	20.0	18.0	13.0	20.0

Table 2: Data of the fractionation pattern produced by actinomycete isolate No. 15

	Mean values of inhibition zones (mm)				
Fractionation pattern	*Micrococcus luteus,* ATCC 9341	*Escherichia coli,* NCTC 10416	*Ps. aeruginosa,* ATCC 10145	*Candida albicans,* IMRU 3669	*Asp. flavus,* IMI 111023
1:15	0.0	0.0	0.0	0.0	0.0
16	12.0	16.0	19.0	12.0	0.0
17	14.0	18.0	23.0	14.5	13.0
18	16.0	21.0	26.0	17.5	16.0
19	18.0	23.0	28.5	18.5	17.0
20	20.0	24.0	30.0	21.0	19.0
21	17.0	22.0	28.0	18.5	17.0
22	16.0	20.0	25.0	15.5	14.0
23	14.0	18.0	22.5	11.0	11.0
24	11.0	14.0	17.0	0.0	0.0
25:50	0.0	0.0	0.0	0.0	0.0

Table 3: Data of the fractionation pattern produced by actinomycete isolate No. 17

	Mean values of inhibition zones (mm)				
Fractionation pattern	*Micrococcus luteus,* ATCC 9341	*Escherichia coli,* NCTC 10416	*Ps. aeruginosa,* ATCC 10145	*C. albicans,* IMRU 3669	*Asp. flavus,* IMI 111023
1:19	0.0	0.0	0.0	0.0	0.0
20	12.0	0.0	0.0	0.0	0.0
21	15.0	0.0	0.0	12.0	0.0
22	20.0	14.0	14.5	12.5	0.0
23	22.0	16.0	16.5	14.5	0.0
24	23.0	18.0	18.5	16.5	11.5
25	21.0	15.0	15.5	14.5	0.0
26	17.0	12.0	12.5	11.5	0.0
27:50	0.0	0.0	0.0	0.0	0.0

Table 3 represents the most active fractions against the tested organisms for actinomycete isolate No. 17 which ranged between fractions No. 20 to 26, that contains the active compounds for further tested. The most active fraction is No. 24 which contains the high concentration for antimicrobial agent the tested microorganisms. The inhibition diameter ranged between 11.5 and 23 mm, respectively. These fractions were collected and concentrated separately under reduced vacuum on a rotary evaporator, kept in a refrigerator till used for further study including chemical tests, elemental analysis and spectrophotometric analysis.

Characterization and biological activities of the antimicrobial agent(s) biosynthesized by actinomycete isolates 15 and 17

Bioautography of the purified antimicrobial agent: Twenty microliters of the purified preparation No. 20 and 24 of the antimicrobial agents produced by actinomycete isolates 15 and 17, respectively were loaded on to paper chromatographic strips in order to test the best condition and solvent used for solubility and for further purification of these fractions. Migration of the antimicrobial agents on paper chromatographic strips was recorded using different solvent systems and their R_f values were calculated as suggested by Bilinov and Khoklov (1970). Data are illustrated in Table 4.

Table 4: The R_f values of the bio-autography of antimicrobial agent(s) produced actinomycete isolates 15 and 17

Developing solvent systems and symbols	R_f value for 15	R_f value for 17
A- Petroleum ether	0.0	0.00
B-Chloroform	0.8	0.70
C-n-Butanol	0.5	0.60
D-Ethyl acetate	1.0	1.00
E-Chloroform-Ethyl acetate (1:1,v/v)	0.9	0.80
F-Chloroform-n-Butanol (1:1, v/v)	0.7	0.60
G-Methanol	0.5	0.70
H-Acetone	0.5	0.20
I-Distilled water	0.0	0.00
K-Ammonium chloride (3%)	0.0	0.00
L-n-Butanol : pyridine : water (2.0.0.6:1.0, v/v)	0.3	0.50
M-n-Butanol : acetic acid : water (1:1:1, v/v)	0.3	0.55

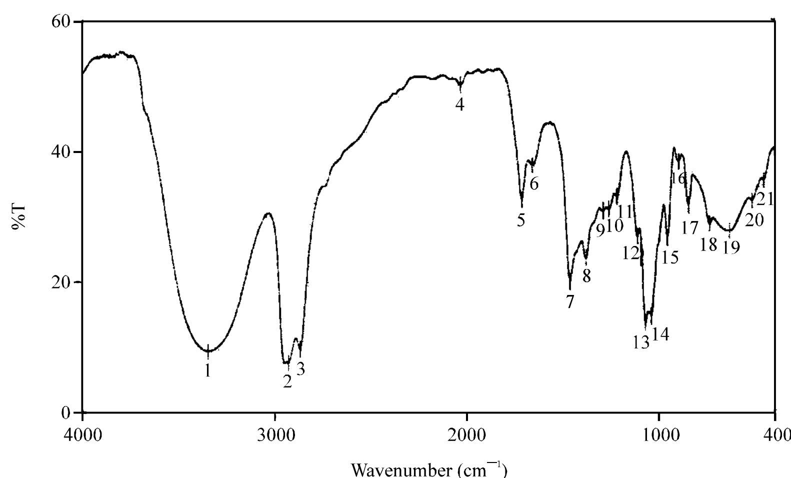

Fig. 1: IR spectrum of antimicrobial agent produced by actinomycete isolate No. 15

Physicochemical characteristics of the purified antimicrobial agent(s): The purified antimicrobial agent produced by actinomycete isolate No. 15 is produces characteristic odour, their melting points are 205°C. The compound is freely soluble in chloroform, ethyl acetate, n-butanol, acetone, ethyl alcohol, methanol and 10% isopropyl alcohol, but insoluble in petroleum ether, hexan and benzene. The purified antimicrobial agent produced by actinomycete isolate No. 17 is produces characteristic odour; their melting points are 115°C. The compound is freely soluble in chloroform, ethyl acetate, n-butanol, acetone, ethyl alcohol, methanol and 10% isopropyl alcohol, but insoluble in petroleum ether, hexan and benzene.

Spectroscopic characteristics: To characterized the purified antimicrobial fractions isolated by actinomycete isolate No. 15 and 17, a spectroscopic analysis was done using infrared (IR) and ultraviolet (UV) analysis tests. Data of IR spectrum on purified fraction produced by actinomycete isolates No. 15, showed characteristic band corresponding to 20 peaks (Fig. 1). Figure 1 explain the presence of at least 20 different compounds by IR spectrum. On the other hand, the UV absorption spectrum of the antimicrobial agent are recorded a maximum absorption peak at 220 and

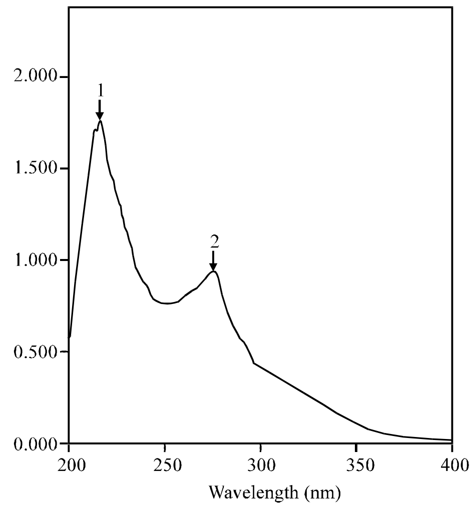

Fig. 2: Ultraviolet absorbance of antimicrobial agent produced by actinomycete isolate No. 15

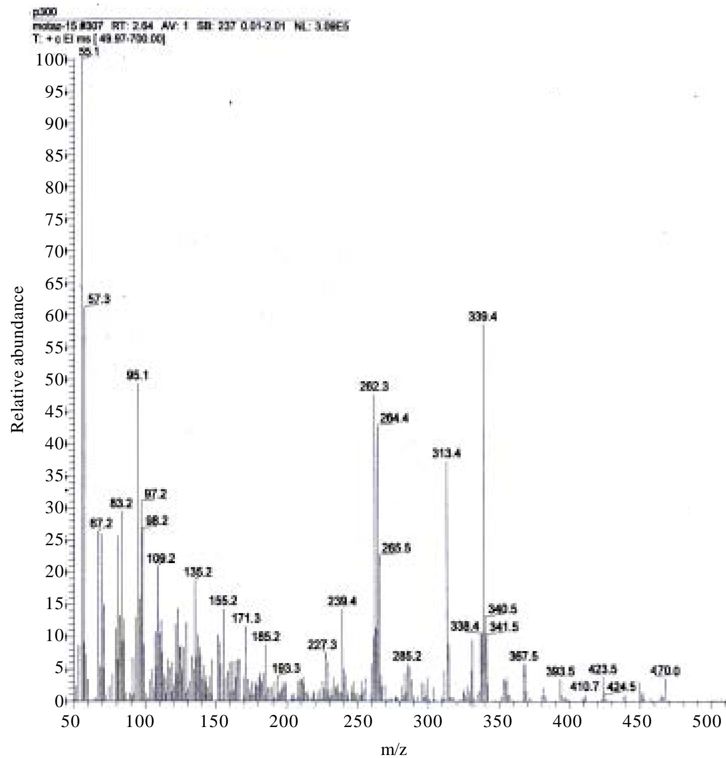

Fig. 3: Mass spectrum of antimicrobial agent produced by actinomycete isolate No. 15

275 nm (Fig. 2). The Mass spectrum showed that the molecular weight at 470.47 (Fig. 3) that is clear it is a cyclic compound mainly with characterized absorption at uv spectrum. The spectroscopic analysis of the purified of antimicrobial agent produced by actinomycete isolate No. 17, indicated that IR spectrum showed a characteristic band corresponding to 19 peaks (Fig. 4). The UV absorption spectrum of the antimicrobial agent are recorded a maximum absorption peak at 235 and 360 nm (Fig. 5). The Mass spectrum showed that the molecular weight at 363. 45 (Fig. 6).

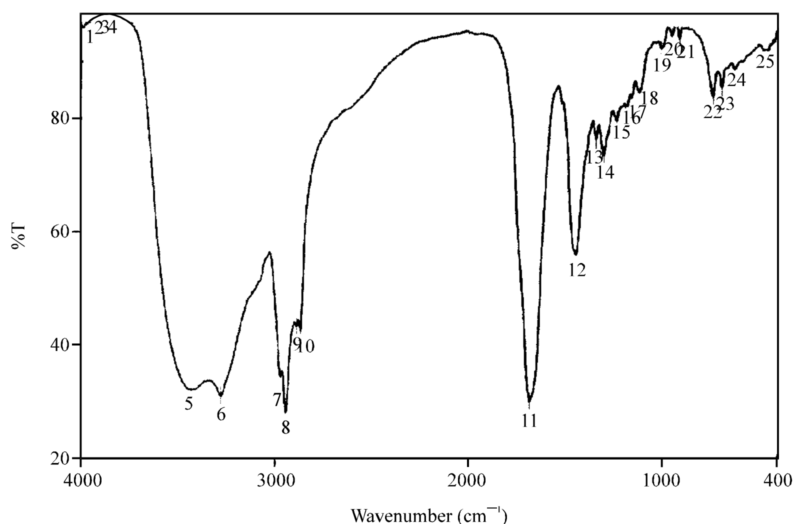

Fig. 4: IR spectrum of antimicrobial agent produced by actinomycete isolate No. 17

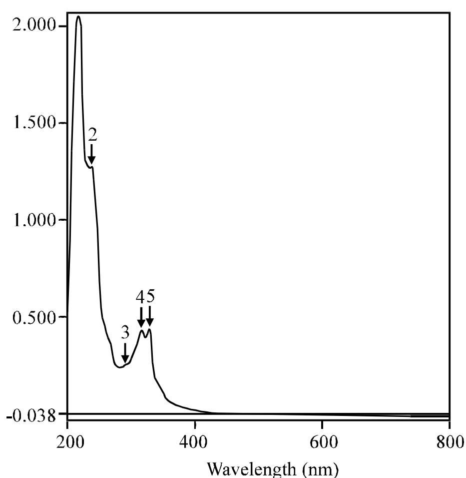

Fig. 5: Ultraviolet absorbance of antimicrobial agent produced by actinomycete isolate No. 17

Elemental analysis: The elemental analytical data of the antimicrobial agent produced by actinomycete isolate No. 15 and 17 showed the following:

C = 45.95; H = 7.28; N = 5.95, O = 40.81 and S = 0.0, with an empirical formula of: $C_{18}H_{34}N_2O_{12}$

The elemental analytical data of the antimicrobial agent produced by actinomycete isolate No. 17 showed the following:

C = 66.09; H = 8.04; N = 3.85, O = 22.01 and S = 0.0, with an empirical formula of: $C_{20}H_{29}NO_5$

Reaction of the antimicrobial agent(s) with certain chemical tests: In order to determine the natural of functional group contains in the purified active fractions, some tests were done to check the presence of carbohydrate molecule, protein moieties, ketons group, or lipid structure.

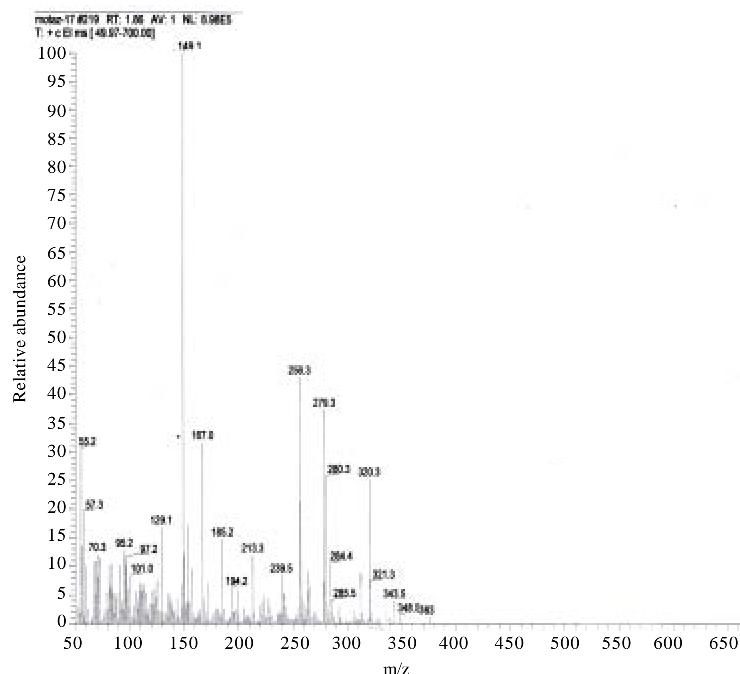

Fig. 6: Mass spectrum of antimicrobial agent produced by actinomycete isolate No. 17

Table 5: Response of the antimicrobial agent produced by actinomycete isolate No. 15 to certain biochemical reactions to reference antibiotic (Mezanomycein)

Chemical test	Result	Remark
Molish's reaction	-	Absent of sugar moiety
Fehling test	-	Absence of free aldehyde or keto sugar
Ninhydrin test	+	Present of free-NH_2 group
Sakaguchi reaction	-	Arginin is Absence
Nitroprusside reaction	-	Absence of Sulfur
Ferric chloride reaction	+	Present of Di-ketons group
Ehrlish reaction	-	Absence of indolic acid
Mayer reaction	+	Presence of nitro group

Presence of some active groups in the fraction may help in expectation of active compound behaviours.

Active purified fraction produced by actinomycete isolate No. 15 indicating the presence of an amino group where ninhydrin, ferric chloride and Mayer tests are positive as indicated in Table 5.

Active purified fraction produced by actinomycete isolate No. 17 indicating the presence of an amide group where only, ferric chloride and Mayer tests are positive; this is clearly indicated that there is no any amino groups as indicated in Table 6.

Identification of the antimicrobial agent(s): On the basis of the recommended keys for the identification of antibiotics (Berdy, 1980a-c; Umezawa, 1967) and in view of the comparative study of the recorded properties of the antimicrobial agent(s), it could be stated that the antimicrobial

Table 6: Response of the antimicrobial agent produced by actinomycete isolate No. 17 to certain biochemical reactions to reference antibiotic (Anthracidin-A)

Chemical test	Result	Remark
Molish's reaction	-	Absence of sugar moiety
Fehling test	-	Absence of free aldehyde or keto sugar
Ninhydrin test	-	Absence of free-NH_2 group
Sakaguchi reaction	-	Arginin is Absence
Nitroprusside reaction	-	Absence of Sulfur
Ferric chloride reaction	+	Present of Di-ketons group
Ehrlish rection	-	Absence of indolic acid
Mayer reaction	+	Presence of nitro group

Table 7: A comparative study of the characteristic properties of the antimicrobial agent produced by actinomycete isolate (15) in relation to Reference antibiotic (Mezanomycein)

Characteristic	Purified antimicrobial agent	Mezanomycein
Melting point	205°C	205°C
Molecular weight	470.47	470.44
Chemical analysis		
C	45.95	45.95
H	7.28	6.86
N	5.95	2.98
O	40.81	44.21
S	0.0	ND
Ultra violet	220 and 275	222 and 276

Table 8: A comparative study of the characteristic properties of the antimicrobial agent produced by actinomycete isolate (17) in relation to Reference antibiotic (Anthracidin-A)

Characteristic	Purified antimicrobial agent	Anthracidin-A
Melting point	115°C	114-116°C
Molecular weight	363.45	363.44
Chemical analysis		
C	66.09	66.09
H	8.04	8.04
N	3.85	3.85
O	22.01	22.01
S	0.0	No data
Ultra violet	235 and 360	233 and 358

agent produced by actinomycete isolate No. 15 is suggestive of being belonging to Mezanomycin antibiotic (Table 7) and the antimicrobial agent produced by actinomycete isolate No. 17 is suggestive of being belonging to Anthracidin-A antibiotic (Table 8).

DISCUSSION

In the late 1960s, the need for new antibiotics began to be questioned on the basis of their medical need, subsequently, the industrial emphasis on antibacterial (synthetic) and antibiotic (natural product-derived) agents changed from being a therapeutic mainstay in most pharmaceutical companies to becoming a low priority area of research (Projan, 2003).

Actinomycetes had been recognized as the potential producers of metabolites such as antibiotics, growth promoting substances for plants and animals, immunomodifiers, enzyme inhibitors and many other compounds of use to man. They had provided about two-thirds (more than (4000) of the naturally occurring antibiotics discovered, including man of those important in medicine, such as aminoglycosides, anthracyclines chloramphenicol, B-lactam, macrolides, tetracyclines-etc. (Gupte *et al.,* 2000). In addition actinomycetes are useful biological tools producing antimicrobials (Gupte *et al.,* 2000).

Streptomycetes are the source of several useful antibiotics that are used not only in the treatment of various human and animal diseases but also in agriculture and biochemistry as metabolic poisons (Jones, 2000). Discovery of new antibiotics produced by streptomycetes, still continues, such as demethyltetracycline produced by *S. aureofaciens* (Ronald, 1996), pyrroindomycins produced by *S. rugosporus* (Abbanat *et al.,* 1999) and meropromycin produced by newly isolated *Streptomyces* sp. Strain MAR01 (EL-Naggar, 2007).

The aim of this study is the biosynthesis of bioactive material from two strains of actinomycetes previously identified as *Streptomyces roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA17 (Al- Humiany, 2006). The two isolates (TA15 and TA17) were grown in sporulating medium composed of starch, potassium nitrate of pH 7 and incubated in a rotary shaker of 250 rpm for 3 days at 30°C. These isolates then used for scaling up of the optimal production of antibiotics in fermetor.

The active metabolites of both *roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA17 were extracted by using different organic solvents. From the tested solvents used for extraction, it was found that ethyl acetate was the best solvent for the extraction of the active substance from the fermented broth of *Streptomyces roseodistaticus* TA 15 and *Streptomyces lavendofoliae* TA17.

Augustine *et al.* (2005) stated that in case of *S. albidoflavus* PU23, maximum antibiotic yield was observed in residue, which was extracted by using n-hexan. Hadjira *et al.* (2005) showed that n-butanol was the most appropriate for antibiotic extraction. The clear filtrate was adjusted at pH 7.0 and extraction process was carried out using ethyl acetate, which was considered to be the best solvent for metabolites of isolates numbers (15 and 17) at the level of 1:1 (v/v), respectively. The organic phase was collected, evaporated under reduced pressure by using a rotary evaporator. The evaporation was continued until viscous syrup was obtained. The residual syrup was dissolved in the least amount of solvent and filtered through Whatman No. 1 filter paper.

The extract (s) were subjected to TLC, bands were detected by using ultraviolet. A band at $R_f = 0.55$ for the compound produced by *Streptomyces roseodistaticus* TA15 and band at $R_f = 0.8$ for the compound produced by *Streptomyces lavendofoliae* TA17. The extract (s) were then subjected to purification by using silica gel column chromatography. The antimicrobial activity was measured by paper disk method and the obtained results indicated that the most active fractions against the tested organisms ranged between 16 to 24 for *Streptomyces roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA17, respectively and the most active fractions against the tested organisms ranged between 20 to 26 for the *Streptomyces lavendofoliae* TA17. Then the active fractions were eluted, collected and concentrated. Many workers used this method such as Tsukamoto *et al.* (2000), Honda *et al.* (2001), Abe *et al.* (2002), Ueno *et al.* (2002) and El-Naggar (2007).

Bioautograhy of the puriefied antimicrobial agents produced by both *Streptomyces roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA 17 were conducted basd on the technique of Weinstein and Wagman (1978). This highly specified technique ws used for the detection of the

active fractions and to determine the R_f values of the antimicrobial agents and their mobility on paper strip chromatography using different solvent system which was suggested by Bilinov and Khokhlov (1970). One definite inhibition zone was detected which revealed that, the antimicrobial agent(s) under study was composed of one spot referring to one pure compound.

The physico-chemical characteristics of the purified two antimicrobial agents revealed that the antimicrobial agents produced by both *Streptomyces roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA 17 were produced characteristic odor, melting point were 205 and 115°C, respectively. The two compounds were freely soluble in chloroform, ethyl acetate, n-butanol, acetone, ethyl alcohol, methanol and 10% isoprppyl alcohol, but insoluble in petroleum ether and benzene.

The spectroscopic analysis of the purified antimicrobial agent produced by *Streptomyces roseodistaticus* TA15 revealed that, the infrared (IR) spectrum showed characteristic band corresponding to 20 peaks. The ultraviolet (UV) absorption spectrum of the antimicrobial agent recorded a maximum absorption peak at 220 and 275 nm). The Mass spectrum showed that the molecular weight at 470.47.

The spectroscopic analysis of the purified of antimicrobial agent produced by *Streptomyces lavendofoliae* TA17, the infrared (IR) spectrum showed characteristic band corresponding to 19 peaks. The ultraviolet (UV) absorption spectrum of the antimicrobial agent recorded a maximum absorption peak at 235 and 360 nm. The Mass spectrum showed that the molecular weight at 363.45.

The elemental analytical data of the antimicrobial agent produced by *Streptomyces roseodistaticus* TA15 showed the following:

C = 45.95; H = 7.28; N = 5.95, O = 40.81 and S = 0.0, with an empirical formula of: $C_{18}H_{34}N_2O_{12}$

The elemental analytical data of the antimicrobial agent produced by *Streptomyces lavendofoliae* TA 17 showed the following:

C = 66.09; H = 8.04; N = 3.85, O = 22.01 and S = 0.0, with an empirical formula of : $C_{20}H_{29}NO_5$

The identification of the antimicrobial agent (s) was carried out on the basis of the recommended keys for the identification of antibiotics and in view of the comparative study of the recorded properties of the antimicrobial agent (s) it could be stated that the antimicrobial agent produced by *Streptomyces roseodistaticus* TA15 is suggestive of being belonging to Mezanomycin antibiotic and the antimicrobial agent produced by *Streptomyces lavendofoliae* TA17 is suggestive of being belonging to Anthracidin-A antibiotic. We suggested the name "Taificidin 1" for the antimicrobial agent who extracted from *St. roseodistaticus* TA15 and "Taificidin 2" for the antimicrobial agent which extracted from *St. lavedofoliae* TA17 as two active compounds isolated for first time from EL-Taif region, KSA, showed remarkable activity against Gram positive, Gram negative and unicellular fungi, with no recorded activity against the tested viruses.

CONCLUSION

- Aim of this study is the biosynthesis of bioactive material from two strains of actinomycetes previously identified as *Streptomyces roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA17

- The two isolates (TA15 and TA17) were grown in sporulating medium composed of starch, potassium nitrate of pH 7 and incubated in a rotary shaker of 250 rpm for 3 days at 30°C. These isolates then used for scaling up of the optimal production of antibiotics in fermetor
- The active metabolites of both *roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA17 were extracted by using different organic solvents. From the tested solvents used for extraction, it was found that ethyl acetate was the best solvent for the extraction of the active substance from the fermented broth of *Streptomyces roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA17
- The extract (s) were subjected to TLC using solvent system composed of chloroform and methanol of the ratio (24:1) (v/v). The bands were then detected by using ultraviolet. The TLC under UV showed band at $R_f = 0.55$ for the compound produced by *Streptomyces roseodistaticus* TA15 and band at $R_f = 0.8$ for the compound produced by *Streptomyces lavendofoliae* TA17. The extract(s) were then subjected to purification by using silica gel column chromatography
- The antimicrobial activity was measured by paper disk method and the obtained results indicated that the most active fractions against the tested organisms ranged between 16 to 24 for *Streptomyces roseodistaticus* TA 15 and *Streptomyces lavendofoliae* TA17 and the most active fractions against the tested organisms ranged between 20 to 26 for the *Streptomyces lavendofoliae* TA17. Then the active fractions were eluted, collected and concentrated
- The physicochemical characteristics of the purified two antimicrobial agents revealed that the antimicrobial agents produced by both *Streptomyces roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA17 were exihabited, melting point at 205 and 115°C, respectively. The two compounds were freely soluble in chloroform, ethyl acetate, n-butanol, acetone, ethyl alcohol, methanol and 10% isoprppyl alcohol, but insoluble in petroleum ether and benzene
- The spectroscopic analysis of the purified antimicrobial agent produced by *Streptomyces roseodistaticus* TA15 revealed that, infrared (IR) spectrum showed characteristic band corresponding to 20 peaks. The ultraviolet (UV) absorption spectrum of the antimicrobial agent recorded a maximum absorption peak at 220 and 275 nm). The Mass spectrum showed that the molecular weight at 470.47. The spectroscopic analysis of the purified of antimicrobial agent produced by *Streptomyces lavendofoliae* TA17, IR spectrum showed characteristic band corresponding to 19 peaks. The UV absorption spectrum of the antimicrobial agent recorded a maximum absorption peak at 235 and 360 nm. The Mass spectrum showed that the molecular weight at 363.45
- The elemental analytical data of the antimicrobial agent produced by *Streptomyces roseodistaticus* TA15 is:

C = 45.95; H = 7.28; N = 5.95, O = 40.81 and S = 0.0, with an empirical formula of : $C_{18}H_{34}N_2O_{12}$

While elemental analytical data of the antimicrobial agent produced by *Streptomyces lavendofoliae* TA17 is:

C = 66.09; H = 8.04; N = 3.85, O = 22.01 and S = 0.0, with an empirical formula of : $C_{20}H_{29}NO_5$

- The identification of the antimicrobial agent (s) was carried out on the basis of the recommended keys indicated that antimicrobial agent produced by *Streptomyces roseodistaticus* TA15 is belonging to Mezanomycin antibiotic and the antimicrobial agent produced by *Streptomyces lavendofoliae* TA17 is belonging to Anthracidin-A antibiotic

- The name "Taificidin1" and "Taificidin2" for the antimicrobial agent which extracted from *St. roseodistaticus* TA15 and from *St. lavedofoliae* TA17, respectively, as two active compounds isolated for first time from EL-Taif region, KSA
- Taificidin 1 and Taificidin 2 showed a remarkable activity against Gram positive, Gram negative and unicellular fungi, with no recorded activity against the tested viruses

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