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Sparsomycin Antibiotic Production by *Streptomyces* Sp. AZ-NIOFD1: Taxonomy, Fermentation, Purification and Biological Activities

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Abstract: This work was carried out in the course of a screening program for specific the bioactive substances that demonstrated inhibitory affects against prokaryotic and eukaryotic microorganisms from actinomycetes strains..Five actinomycete isolates could be isolated from water sample collected from River Nile, Egypt were screened for antimicrobial activities in starch nitrate medium. One of the actinomycete cultures (AZ-NIOFD$_1$) was found to produce a wide spectrum antimicrobial agent. The most potent of the producer strains was selected and identified. The cultural, physiological and biochemical characteristics of the isolate identified the strain as a member of the genus *Streptomyces.* The nucleotide sequence of the 16S rRNA gene (1.5 kb) of the most potent strain evidenced a 98% similarity with *S. tritolerans* and a 96% similarity with *S. plicatus* 16_S rRNA genes and the isolated strain was ultimately identified as *Streptomyces* sp. MAR01. The extraction of the fermentation broth of this strain resulted in the isolation of one major compound, which was active *in vitro* against Gram-positive, Gram-negative bacteria and unicellular and filamentous fungi. The physico-chemical characteristics of the purified antibiotic *viz*. color, melting point, solubility, elemental analysis, spectroscopic characteristics and chemical reactions have been investigated. This analysis indicates a suggested imperical formula of $C_{13}H_{21}N_3O_6S_2$. The minimum inhibition concentrations "MICs" of the purified antibiotic were also determined. In conclusion, the collected data emphasized the fact that the purified antibiotic compound was suggestive of being belonging to sparsomycin antibiotic produced by *Streptomyces violaceusniger*, AZ-NIOFD$_1$.

Key words: Sparsomycin antibiotic, *Streptomyces violaceusniger*, 16s rRNA, Taxonomy, Fermentation, Purification and Biological activities

INTRODUCTION

Natural organic compounds produced by microorganisms are an important screening target for a variety of bioactive substances [1]. Compound of actinomycete origin, in particular are valuable in the field of bioactive Natural products [2]. However, the rate of discovery of novel substances from microorganisms, especially from actinomycetes of terrestrial origin, has recentaly decreased [3]. Filamentous soil bacteria belonging to the genus *Streptomyces* are rich sources of a higher number of bioactive natural products with biological activity extensively used as pharmaceuticals and agrochemicals [4]. The sparsomycin antibiotic is a universal translation inhibitor that blocks protein synthesis in prokaryotic and eukaryotic cell [5]. The drug binds and causes important conformational changes in the peptidyl transferase active center. Thus, it was found that sparsomycin can block the binding of substrates at the A-site (24) but it enhances binding to the P-site [6]. Recently, this antibiotic was found to interact with nucleotide A2602 in the peptidyl transferase center of the bacterial ribosome [7]. The drug was initially developed as a potential antitumor agent, although toxicity soon limited its clinical application [8]. Sparsomyin is produced by *Streptomyces sparsogenes*, which is obviously resistant to the drug [9]. The molecular weight of sparsomycin antibiotic is 361 and empirical formula $C_{13}H_{21}N_3O_6S_2$ [10].

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The present work was carried out to describe the isolation of an actinomycete strain from water sample collected from River Nile Egypt, which generates an antimicrobial compound. The identification of this strain, based on the cultural, morphology, physiology and biochemical characteristics, as well as 16s rRNA methodology, is also reported. The primary bioactive substance was isolated, purified and its biological activities were determined.

MATERIALS AND METHODS

Microorganisms and Culture Conditions: Five actinomycete strains could be isolated from water sample collected from River Nile, Egypt. Actinomycetes were isolated using Starch-nitrate agar. The plates were incubated for 5 days at 30°C. The isolated actinomycete strains were then screened with regard to their potential to generate bioactive compounds. The most potent producer strains was then selected and identified. The cultures were maintained on starch-nitrate agar slants. The inoculated agar medium was incubated for 5 days at 30°C, then maintained at 4°C until further use.

Screening for Antimicrobial Activity: The anti-microbial activity was determined by cup method assay [11].

Taxonomic Studies of Actinomycete Isolate: Morphological characteristics of the most potent produce strain AZ-NIOFD$_1$ grown on starch nitrate agar medium at 30°C for 8 days was examined under light and scanning electron microscopy (JEOL Technics Ltd.). Physiological and biochemical characteristics: lecithinase was conducted on egg–yolk medium [12]. Also, lipase [13], Protease [14], pectinase [15], á-amylase [16] and Catalase test [17] were monitored using standard methods. Also, Melanin pigment [18]. Degradation of both esculin and xanthine [19], Nitrate reduction [20]. Hydrogen sulphide production, citrate utilization, coagulation of milk and oxidase test [16] as well as the utilization of different carbon and nitrogen sources [21] were performed. Cell wall was performed by the method of Becker *et al.* [22] and Lechevalier and Lechevalier [23]. The cultural characteristics were studied in accordance with the guidelines established by the International *Streptomyces* Project [24]. Colors characteristics were assessed on the scale developed by Kenneth and Deane [25].

DNA Isolation and Manipulation: The locally isolated actinomycete strain was grown for 7 days on a starch agar slant at 30°C. Two ml of a spore suspension were inoculated into the starch- nitrate broth and incubated for 3 days on a shaker incubator at 200 rpm and 30°C to form a pellet of vegetative cells (pre-sporulation). The preparation of total genomic DNA was conducted as described by Sambrook *et al.* [26].

Amplification and Sequencing of the 16S rRNA Gene: PCR amplification of the 16S rRNA gene of the local actinomycete strain was conducted using two primers, StrepF; 5.-ACGTGTGCAGCCCAAGACA-3. and Strep R; 5.ACAAGCCCTGGAAACGGGGT-3, [27]. The PCR mixture consisted of 30 pmol of each primer, 100 ng of chromosomal DNA, 200 µM dNTPs and 2.5 units of Taq polymerase, in 50 µl of polymerase buffer. Amplification was conducted for 30 cycles of 1 min at 94°C, 1 min of annealing at 53°C and 2 min of extension at 72°C. The PCR reaction mixture was then analyzed via agarose gel electrophoresis and the remaining mixture was purified using QIA quick PCR purification reagents (Qiagen, USA). The 16S rRNA gene was sequenced on both strands via the dideoxy chain termination method [28]. The 16S rRNA gene (1.5 kb) sequence of the PCR product was acquired using a Terminator Cycle Sequencing kit (ABI Prism 310 Genetic Analyzer, Applied Biosystems, USA).

Sequence Similarities and Phylogenetic Analysis: The BLAST program (www.ncbi.nlm.nih.gov/blst) was employed in order to assess the degree of DNA similarity. Multiple sequence alignment and molecular phylogeny were evaluated using BioEdit software [29]. The phylogenetic tree was displayed using the TREE VIEW program.

Fermentation: A loopful of the, *Streptomyces violaceusniger*, AZ-NIOFD$_1$ from the 5-day culture age was inoculated into 250 ml Erlenmeyer flasks containing 75 ml of liquid starch nitrate medium (seven flasks). The flasks were incubated on a rotary shaker (200 rpm) at 30°C for 5 days. Twenty-liter total volume was filtered through Whatman No.1 filter paper, followed by centrifugation at 5000 r.p.m at 10°C for 20 minutes.

Extraction: The clear filtrate was adjusted at different pH values (4 to 9) and extraction process was carried out using different solvents separately at the level of 1:1 (v/v).

The organic phase was concentrated to dryness under vacuum using a rotary evaporator at a temperature not exceeding 50°C.

Precipitation: The precipitation process of the crude compound was carried out using petroleum ether (b.p 60-80°C) followed by centrifugation at 5000 r.p.m 10°C for 15 min.

Purification by TLC: Separation of the antimicrobial compound into its individual components was conducted by thin layer chromatography using chloroform and methanol (24: 1, v/v) as a solvent system.

Purification by Column Chromatography: The purification of the antimicrobial compound was carried out using silica gel column (2.5 X 50) chromatography. Chloroform and Methanol 8:2 (v/v), was used as an eluting solvent. Fifty fractions were collected (each of 5 ml) and tested for their antimicrobial activities [30].

Physico-Chemical Properties of Antimicrobial Agent:
Elemental Analysis: The elemental analysis C, H, O, N and S ratio was carried out at the microanalytical center, Cairo University, Egypt.

Spectroscopic Analysis: The IR, UV, Mass spectrum, NMR spectrum and HPLC-spectrum were determined at the micro analytical center of Cairo University, Egypt.

Reaction of the Antimicrobial Agent with Certain Chemical Test: For this purpose, the following reactions were carried out: Molish's, Fehling, Sakaguchi, ninhydrin, Ehrlish, Nitroprusside, Ferric chloride and Mayer reactions [30].

Biological Activity: The minimum inhibitory concentration (MIC) has been determined by the cup method assay [11].

Characterization of the Antimicrobial Agent: The antibiotic produced by *Streptomyces violaceusniger*, AZ-NIOFD$_1$ was identified according to the recommended international references of [10, 31- 34].

RESULTS

Screening for the Antimicrobial Activities: One of the actinomycete culture AZ-NIOFD1 from five cultures was found to exhibit various degrees of activities against Gram-positive and Gram-negative bacteria and unicellular and some filamentous fungi (Table 1).

Identification of the Actinomycete Isolate: The vegetative mycelia grew abundantly on both synthetic and complex media and show fragmentation into bacillary elements. The aerial mycelia grew abundantly on starch- nitrate agar medium and Oatmeal agar medium (ISP-3). The Spore chains were spiral and had a smooth surface (Fig. 1).

Fig. 1: Scanning electron micrograph of the actinomycete isolate AZ-NIOFD$_1$ growing on starch nitrate agar medium showing spore chain spiral shape and spore surfaces smooth (X13,000).

Table 1: Antimicrobial potentialities produced by actinomycetes isolated from River Nile, Egypt

	Tested organism										
	*Mean values of inhibition zones (in mm) against										
	Bacteria						Fungi				
Isolate Nos.	*Bacillus subtilis*, NCTC 1040	*Staph. aureus*, NCTC 7447	*Micrococcus luteus*, ATCC 9341	*E. coli* NCTC 10416	*Klebsiella pneumonia*, NCIMB 9111	*Ps.aeruginosa*, ATCC 10145	*Saccharomyces cerevisiae*, ATCC 9763	*Asp. niger* IMI 31276	*Asp fumigatus* ATCC 16424	*Fusarium oxysporum*	*Penicillium. chrysogenum*
NIOFD$_1$	29.0	27.0	26.0	29.0	27.0	28.0	30.0	23.0	24.0	32.0	0.0
NIOFD$_2$	22.0	20.0	19.0	18.0	15.0	15.0	0.0	0.0	0.0	0.0	0.0
NIOFD$_3$	20.0	21.0	19.0	0.0	0.0	0.0	16.0	14.0	15.0	0.0	0.0
NIOFD$_4$	25.0	23.0	21.0	20.0	19.0	21.0	0.0	0.0	0.0	0.0	0.0
NIOFD$_5$	0.0	0.0	0.0	21.0	22.0	20.0	0.0	16.0	17.0	0.0	0.0

•Mean values of 4 determinations

Table 2: Cultural characteristics of the actinomycete isolate, AZ-NIOFD$_1$

Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
1-Starch nitrate agar medium	Good	264-L.Gray Light gray	76-1-y-br Light yellowish brown	-
2-Tryptone yeast extract broth (ISP-1)	No growth	-	-	-
3-Yeast extract malt extract agar medium (ISP-2)	No growth	-	-	-
4- Oat-meal agar medium (ISP-3)	moderate	264-L.Gray Light gray	76-1-y-br Light yellowish brown	-
5-Inorganic salts starch agar medium (ISP-4)	Good	264-L.Gray Light gray	92-y White Yellowish white	-
6-Glycerol – Asparagine agar medium (ISP-5)	Good	264-L.Gray Light gray	76-1-y-br Light yellowish brown	-
7-Peptone yeast extract iron agar medium (ISP-6)	moderate	264-L.Gray Light gray	76-1-y-br Light yellowish brown	58 m-br moderate brown
8-Tyrosine agar medium (ISP-7)	Poor	264-L.Gray Light gray	76-1-y-br Light yellowish brown	58 m-br moderate brown
9- Potato dextrose agar medium	Good	264-L.Gray Light gray	76-1-y-br Light yellowish brown	-
10- Fish meal agar medium	Good	263-White White	76-1-y-br Light yellowish brown	-
11- Glucose casein agar medium	moderate	92-y White Yellowish white	76-1-y-br Light yellowish brown	-

*The color of the organism under investigation was consulted with the ISCC-NBS color –name charts illustrated with centroid color

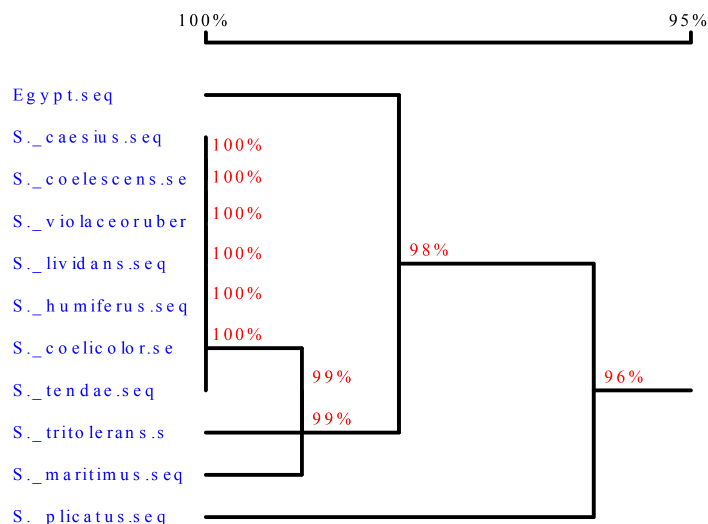

Fig. 2: The phylogenetic position of the local *Streptomyces* sp. strain MAR01 among neighboring species. The phylogenetic tree was based on the pairwise comparisons of 16_S rDNA sequences

Neither both sclerotic granules and sporangia nor flagellated spores were observed. The cell wall hydrolysate contains LL-diaminopimelic acid (LL-DAP) and sugar pattern not detected.

The culture characteristics of actinomycete isolate AZ-NIOFD$_1$ shows the aerial mycelium is gray and rarely white; substrate mycelium is light yellowish brown or light grayish yellow and no diffusible pigment was produced (Table 2).

The physiological and biochemical characteristics of actinomycete isolate AZ-NIOFD$_1$ were summarized in Table 3.

Identification of Actinomycete Isolate, AZ-NIOFD$_1$: This was performed basically according to the recommended international Key's *viz*. and Numerical taxonomy of *Streptomyces* species program. On the basis of the previously collected data and in view of the comparative

Table 3: The physiological and biochemical characteristics of the actinomycete isolate AZ-NIOFD$_1$

Characteristic	Result
Spore mass	Gray
Spore surface	Smooth
Spore chain	Spiral
Color of substrate mycelium	Light yellowish brown
Diffusible pigment	Not produced
Diaminopimelic acid (DAP)	LL-DAP
Sugar Pattern	Not detected
Hydrolysis of:	
Protein and Starch	+
Lipid and Pectin	+
Egg-yolk (lecithin)	-
Catalase test	-
Production of melanin pigment	-
Degradation of: Esculin	+
Degradation of Xanthin	-
H_2S Production	-
Nitrate reduction	+
Citrate utilization	+
Urea test	+
Coagulation of milk	-
Utilization of	
D-Xylose	-
D- Mannose	+
D- Glucose	+
D- Galactose	+
Rhamnose	+
Raffinose	+
Mannitol	+
L- Arabinose	+
meso-Inositol	+
Lactose	+
Maltose	-
Trehalose	+
D-fructose	+
Sucrose	-
Starch	+
L-Cycteine	±
L-Valine	-
L-Histidine	+
L-Phenylalanine	+
L-Hydroxproline	-
L-Lysine	+
L-Arginine	+
L-Serine	+
L-Tyrosine	+
Growth with	
Thallous acetate (0.001)	-
Sodium azide (0.01)	+
Phenol (0.1)	-
Growth temperature	30 °C (20-45 °C)
Optimum pH	7.0 (5.5-7.5)
Resistance to antibiotics	
Ampicillin (10 ug); Ceplalexin (30 ug); Colistin (10 ug); Erthromycin (15 ug), Rifampicin (5 ug) and Amphotericin B(100 ug)	+
Antimicrobial activity against	
Bacillus subtilis, NCTC 1040	+
Staph. aureus, NCTC 7447	+
Saccharomyces cerevisiae ATCC 9763	+
Asp. niger IMI 31276	+

+= Positive, - = Negative and ± = doubtful results.

study of the recorded properties of AZ-NIOFD$_1$ in relation to the most closest reference strain, viz. a 99% similarity with *Streptomyces violaceusniger* (score 0.99), it could be stated that actinomycetes isolate, AZ-NIOFD$_1$ is suggestive of being likely belonging to *Streptomyces violaceusniger,* AZ-NIOFD$_1$.

Molecular Phylogeny: The resulted sequence was aligned with available, almost compete sequence of type strains of family streptomycetaeae and then with corresponding sequences of representative *Streptomyces* sepecies, in each case, the reference sequence was retrieved from the GenBank databases. The phylogenetic tree (diagram) revealed that the local isolate is closely related to *Streptomyces* sp rather than to *S. tritolerans* by a distance of 0.98 (similarity matrix is 98%). It joined *S. plicatus* by a distance of 0.96, where the similarity matrix is 96%) (Fig. 2). According to the obtained results, 16s rDNA sequences, combined with the classical identification system of actinomycetes was useful when discriminating organisms.

Fermentation, Extraction and Purification: The fermentation process was carried out for five days at 30°C using liquid starch nitrate as production medium. Twenty-liter total volume filtered was conducted followed by centrifugation at 5000 r.p.m. at 10°C for 20 minutes. The clear filtrates containing the active metabolite (20 liters), was adjusted to pH 7.0 then extraction process was carried out using n-Butanol at the level of 1:1 (v/v). The organic phase was collected and evaporated under reduced pressure using rotary evaporator. The antimicrobial compound was precipitated by petroleum ether (b.p. 60-80°C) and centrifuged at 5000 r.p.m at 10°C for 15 minute. Separation of antimicrobial agent into individual components was carried out by thin-layer chromatography using a solvent system composed of chloroform and methanol (24: 1, v/v). Only one band at R_f = 0.75 showed antimicrobial activity. The purification process through column chromatography packed with silica gel and an eluting solvents composed of chloroform and methanol (8:2, v/v), revealed that the most active fractions are fractions Nos. 23 to 30.

Physicochemical Characteristics: The purified antimicrobial agent is soluble in chloroform, n-Butanol, acetone, carbon tetrachloride, ethanol, DMSO and methanol but insoluble in petroleum ether, hexane and benzene with a melting point of 205°C.

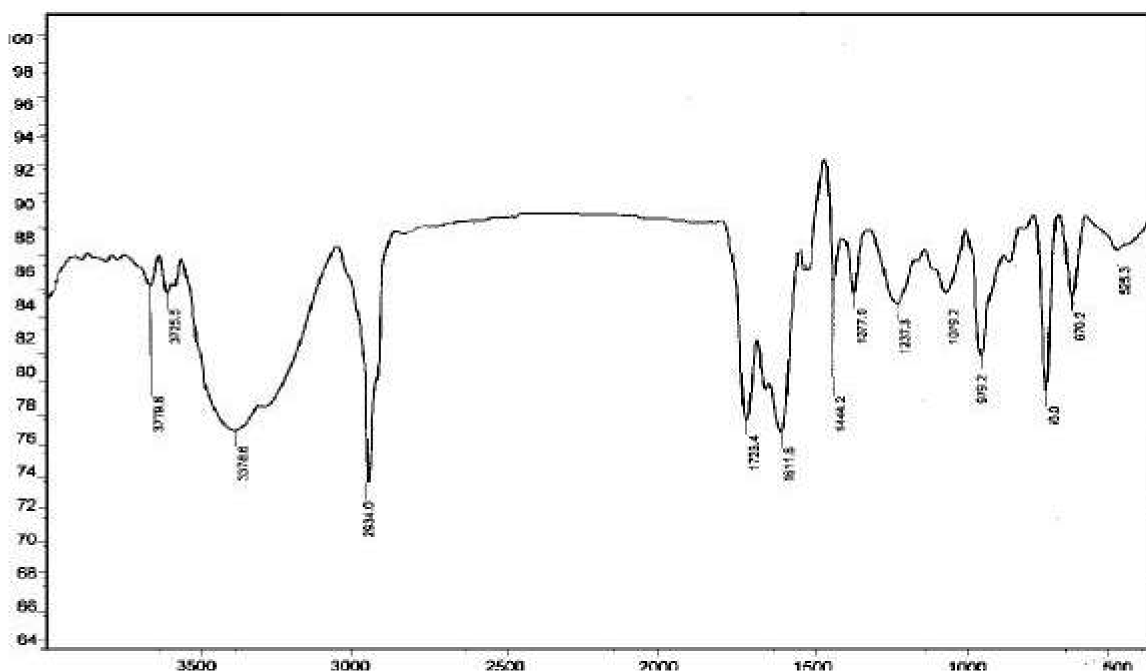

Fig. 3: Infrared spectrum of the antimicrobial agent

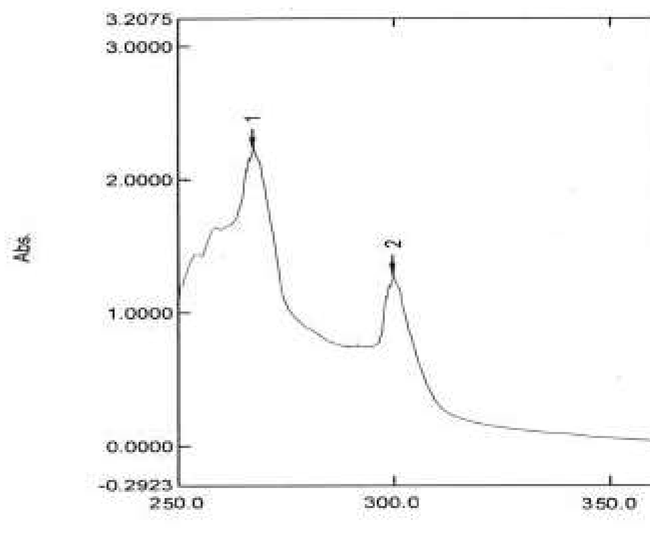

Fig. 4: Ultraviolet absorbance of antimicrobial agent

Elemental Analysis: The elemental analytical data of the antimicrobial agent revealed the following data: C=41.71; H=5.24; N= 10.95, O = 36.1 and S= 16.95. This analysis indicates a suggested calculated imperical formula of $C_{13}H_{21}N_3O_6.S_2$.

Spectroscopic Characteristics: The infrared (IR) spectrum of the antimicrobial agent showed characteristic band corresponding to 23 peaks (Fig.3).The ultraviolet (UV) absorption spectrum of the antimicrobial agent recorded a maximum absorption peak at 270 and 302 nm (Fig. 4). The Mass spectrum revealed that the molecular weight is 361.3 (Fig.5).

Biochemical Reaction of the Antimicrobial Agent: The reactions revealed the detection of certain groups in the investigated molecule. The antimicrobial agent exhibited positive results with nitroprusside, ninhydrin, ferric chloride and Mayer tests and negative results with molish's, fehling, sakaguchi and ehrlish reactions.

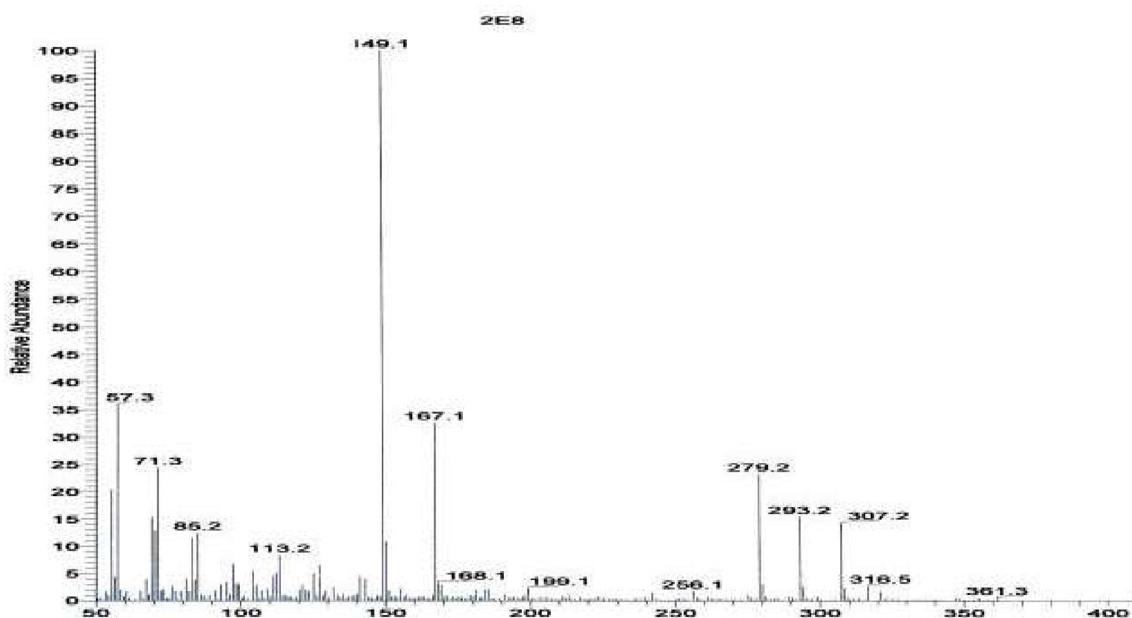

Fig. 5: Mass Spectrum of antimicrobial agent

Table 4: Biological activities (MIC) of the antibacterial agent by cup method assay

Test organisms		MIC (µg/ml) concentrations
A- Bacteria		23.43
Staph. aureus	NCTC 7447	31.25
Micrococcus luteus	ATCC 9341	15.6
Bacillus pumilus	NCTC 8214	11.7
Bacillus subtilis	NCTC 10400	11.7
Escherichia coli	NCTC 10416	15.6
Klebsiella pneumonia	NCTC 9111	15.6
Pseudomonas aeruginosa	ATCC 10415	11.7
B- Fungi		
Saccharomyces cerevisiae	ATCC 9763	15.6
Candida albicans	IMRU 3669	62.50
Asp. niger	IMI 31276	62.50
Asp. fumigatus	ATCC 16424	11.7
Fusarium oxysporum		62.50
Fusarium moniliform		>100
Alternaria alternata		0.0
Botrytis cinerea		0.0
Rhizoctonia solani		62.50

Biological Activities of the Antimicrobial Agent: Data of the antimicrobial agent spectrum indicated that the agent is active against Gram-positive (MIC ranged from 11.7 to 31.25 µg/ml) and Gram-negative bacteria and unicellular

Table 5: A comparative study of the characteristic properties of AZ-NIOFD$_1$ antibiotic in relation to Reference antibiotic (Sparsomycin) [10, 31-34]

Characteristic	Sparsomycin	Purified antibiotic
Melting point	208-209°C	205°C
Molecular weight	361	361.3
Chemical analysis:		
C	41.78	41.71
H	5.20	5.24
N	10.93	10.95
S	16.95	16.95
Ultra violet	270 and 302	270 and 302
Formula	$C_{13}H_{21}N_3O_6S_2$	$C_{13}H_{21}N_3O_6S_2$
Active against	Gram-Positive and Gram-Negative Bacteria and certain fungi	Gram-Positive and Gram-Negative Bacteria and certain fungi

(MIC ranged from 11.7 to 15.6 µg/ml) (MIC ranged from 11.7 to 31.25 µg/ml). On the other hand, the filamentous fungi were most resistant test organisms where MIC showed that more 62.5 µg/ml (Table 4).

Identification of the Antimicrobial Agent: 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, it could be stated that the antimicrobial agent is suggestive of being belonging to Sparsomycin antibiotic) (Table 5).

DISCUSSION

The actinomycete isolate, AZ-NIOFD1 was isolated from water sample collected from River Nile, Egypt The isolate was growing on starch nitrate agar medium for investigating its potency to produce antimicrobial agents. The actinomycete isolate exhibited a wide spectrum antimicrobial agent [1].

Identification process has been carried out [35-37] and Numerical taxonomy of *Streptomyces* species program. For the purpose of identification of actinomycete isolate, the morphological characteristics and microscopic examination emphasized that the spore chain is spiral. Spore mass is light gray and rarely whit; while spore surface is smooth, substrate mycelium is light yellowish brown or light grayish yellow and no diffusible pigment was produced. The present results of physiological, biochemical characteristics and cell wall hydrolysate of actinomycetes isolate, exhibited that the cell wall containing LL-diaminopimelic acid (DAP) and sugar pattern of cell wall hydrolysate could not detected. These results emphasized that the actinomycetes isolate related to a group of *Streptomyces* [36, 37].

In view of all the previously recorded data, the identification of actinomycete isolate AZ-NIOFD$_1$was suggestive of being belonging to *Streptomyces violaceusniger,* AZ-NIOFD$_1$. The resulted sequence was aligned with available almost compete sequence of type strains of family streptomycetaeae. The phylogenetic tree (diagram) revealed that the local isolate is closely related to *Streptomyces* sp rather than to *S. tritolerans* by a distance of 0.98 (similarity matrix is 98%). It joined *S. plicatus* by a distance of 0.96, where the similarity matrix is 96%.

The active metabolites were extracted by n-Butanol at pH 7 [38, 39]. The organic phase was collected and evaporated under reduced pressure using rotary evaporator. The extract was concentrated and treated with petroleum ether (b.p. 60-80°C) for precipitation process. Separation of antimicrobial agent into individual components was carried out by thin-layer chromatography using a solvent system composed of chloroform and methanol (24: 1, v/v) [30]. Only one band at R_f = 0.75 showed antimicrobial activity. The purification process through a column chromatography packed with silica gel and an eluting solvents composed of chloroform and methanol (8:2, v/v), indicated that maximum activity was recorded at fraction Nos. 23 to 30 [40, 41].

The physico-chemical characteristics of the purified antibiotic revealed that, The purified antimicrobial agent is soluble in chloroform, n-Butanol, acetone, carbon tetrachloride, ethanol, DMSO and methanol but insoluble in petroleum ether, hexane and benzene with a melting point of 205°C [4, 10].

A study of the elemental analysis of the antibacterial agent lead to an imperical formula of*:* $C_{13}H_{21}N_3O_6.S_2$. The spectroscopic characteristics of the antimicrobial agent under study revealed the presence of the maximum absorption peak in infrared (IR) spectrum of the antimicrobial agent showed characteristic band corresponding to 23 peaks. The ultraviolet (UV) absorption spectrum recorded a maximum absorption peak at 270 and 302 nm. The Mass spectrum revealed that the molecular weight 361.3 [10, 42].. The biochemical tests of antimicrobial agent exhibited positive results with nitroprusside, ninhydrin, ferric chloride and Mayer tests and negative results with molish's, fehling, sakaguchi and ehrlish reactions [40, 41].

The biological activities (MIC) of the antimicrobial agent emphasized that the antibiotic are active against Gram-positive (MIC ranged from 11.7 to 31.25 µg/ml) and Gram-negative bacteria (MIC ranged from 11.7 to 15.6 µg/ml). On the other hand the filamentous fungi were most resistant test organisms where MIC showed that more 62.5 µg/ml [43].

Identification of antimicrobial agent according to recommended international keys indicated that the antibiotic is suggestive of being belonging to Sparsomycin antibiotic [10, 31-34, 42, 44]. It could be concluded that the sparsomycin antibiotic produced by *Streptomyces violaceusniger, AZ-NIOFD$_1$ that demonstrated inhibi$_t$ory affects against patho$_g$enic microorganisms, Gram positive and G*ram negativ$_e$ bacteria and unicellular and filamentous fungi.

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