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Antibacterial Potential with Molecular Docking Study against Multi-Drug Resistant Bacteria and *Mycobacterium tuberculosis* of Streptomycin Produced by *Streptomyces atroverins*, strain Askar-SH50

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ABSTRACT

The actinobacterium strains were isolated from marine samples that collected from South Sinai Sharm El-Sheikh, Egypt. These isolates were screened for antimicrobial activities in starch nitrate medium. One of the actinomycete isolates (Askar-SH50) was found to produce a broad spectrum antimicrobial activity. Identification of the most potent isolate was performed according to the cell wall chemo-type analysis and spore morphology. From results identification obtained suggested that this strain is a Streptomyces. Further cultural, physiological characteristics and analysis of the nucleotide sequence of 16S rRNA gene evidenced a 99% similarity with Streptomyces atroverins. The isolated strain was eventually identified as Streptomyces atroverins, strain Askar-SH50 and recorded in gene bank with accession number KU740212.The fermentation broth extract of a strain gives one major active compound. The antimicrobial activities of purified active compound showed strong activity against Gram-positive and Gram-negative bacteria. It also, exhibits strong potential towards multi-drug resistant bacteria include Staphylococcus aureus (MRSA), Staphylococcus epidermis, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumonia and Mycobacterium tuberculosis. The results of MIC are listed as: 125 µg/ml, 15.62µg/ml, 3.90 µg/ml, 62.5 µg/ml, 7.81 µg/ml, 3.90 µg/ml and 15.62 µg/ml, respectively. The physicochemical features of the purified antibiotic included solubility, color, melting point, spectroscopic characteristics, elemental analysis, and chemical reactions have been examined. This investigation symbolizes an expected empirical formula of $C_{21}H_{39}N_7O_{12}$. Molecular modeling was performed on streptomycin in order to emphasize its mode of action as an anti TB, and to determine its possible binding interactions with Mycobacterium tuberculosis enoyl-reductase InhA enzyme. In conclusion, the collected data emphasized the fact that the purified antibiotic compound was suggestive of being belonging to streptomycin antibiotic produced by Streptomyces atroverins strain Askar-SH50. Validation of the molecular docking protocol upon Mycobacterium tuberculosis registers that, streptomycin provided from Streptomyces atroverins, strain Askar-SH50 show binds to the amino acid deposits inside the binding pocket among encouraging results.

Keywords: *Streptomyces atroverins* strain Askar-SH50*;* Streptomycin antibiotic; Antimicrobial activity; Multi-drug resistant bacteria*; Mycobacterium tuberculosis;* Molecular docking

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INTRODUCTION

General organic composites generated through microorganisms are an essential screening target for a variety of bioactive substances [1]. Actinomycetes are prokaryotes with remarkably different metabolic possibilities. These are prolific producers of various bioactive compounds and have given over two third of naturally happening antibiotics discovered and maintain to be the primary source of novel and useful compounds such as antibiotics, enzymes, pigments, herbicides, insecticides, and immunomodulators, etc. [2].

Marine actinobacteria have been mentioned upon as possible origins of bioactive composites, and the work done beforehand has confirmed that these microbes are the valuable sources of secondary metabolites. They hold an extended area as quarries in selecting applications due to their diversity and their demonstrated ability to produce novel metabolites and other molecules of pharmaceutical significance [3]. However, the percentage of a discovery of novel materials from microorganisms, especially from actinomycetes of terrestrial origin, has recently decreased [4].

Marine ecosystem is nevertheless an unexplored estuarine environment of its rich microbial variety. There are tremendous opportunities for the existence of possible microbes to resist metal stress in its nutrient breeding habitat. With this experience, we should separate a different *Streptomyces cyaneus* strain Alex-SK121and studied its antimicrobial activity upon a species of pathogenic bacteria and fungi [5]. Filamentous bacteria relating to the genus *Streptomyces* are valuable sources of a higher number of bioactive natural products with biological activity widely used as pharmaceuticals and agrochemicals [6]. Streptomycin is an antibiotic drug, the first of a class of drugs called aminoglycosides to de discovered, and it was the first antibiotic treatment of tuberculosis. It was obtained from the actinobacterium *Streptomyces griseus*. Streptomycin is a bactericidal antibiotic [7]. Aminoglycosides are protein synthesis inhibitors and used to treat various types of bacterial infections [8]. Streptomycin is active against a large number of bacteria found among the Gram-negative, Gram-positive, acid-fast groups and the spirochetes; it has relatively little activity against anaerobic bacteria, fungi, protozoa, and viruses [9]. A variety of human and animal diseases caused by various bacteria respond readily to streptomycin treatment. These included tularemia, urinary tract infections, especially those resistant to sulfa drugs and penicillin, *Klebsiella* and *Hemophilus* infections, bacteremia due to penicillin-resistant organisms, various forms of meningitis, and whooping cough. Streptomycin was also found to be helpful in the treatment of a variety of other diseases, such as leprosy, typhoid fever, brucellosis, certain forms of tuberculosis, and probably also bacillary dysentery and cholera [10].

In addition to the experimental work, the computational work like Molecular modeling of streptomycin inside *Mycobacterium tuberculosis* enoyl-reductase InhA enzyme's active site was performed [11]. Molecular modeling is used to mimic the behavior of molecules inside a specific receptor. After energy minimization of the molecule to be docked, several conformations were generated through the program, and the ligand is docked inside the active site of the enzyme. The best fitting conformation is chosen according to the energy score and amino acids interactions with the introduced ligand [12]. It is used in the field of computational chemistry, drug design, computational biology and materials chemistry to investigate molecular methods varying from inadequate chemical arrangements to large biological molecules.

The aim of this study describes the isolation and identification of *Streptomyces atroverins*. Production, Extraction, purification and characterization of active compound produced by *Streptomyces atroverins*, strain Askar-SH50 and also, investigation of antibacterial activity against multi-drug resistant bacteria and *Mycobacterium tuberculosis* with molecular docking study upon *Mycobacterium tuberculosis*.

EXPERIMENTAL SECTION

Chemicals

All the media components from (Oxide), Chemicals, and reagents used in the following experiments were of analytical grade and used without further purification.

Collection of Samples and isolation of actinomycetes

Marine sediment samples from Sharm El-Sheikh, South Sinai governorate, Egypt were collected in sterile airlock polyethylene bags and stored at 4°C. Selective pre-treatments were performed to increase the number of mycelium forming actinomycetes about the non-actinomycetes heterotrophic microbial flora. The collected samples were air-dried, mixed with $CaCO_3$ and incubated for five days at 37°C, then sieved to remove various unwanted contaminant materials before planting [13]. The sterile plates containing starch nitrate medium were inoculated with serially diluted samples and incubated at 37°C until the appearance of colonies with a strong leathery texture, dry or folded appearance, and branching filaments with or without aerial mycelia [14]. Pure colonies were isolated, and subcultures were carried out by streaking the particular strain directly on ISP-4 agar media.

Preliminary screening for antimicrobial activity

Test microorganisms:

The test microorganisms were used in the present study, Gram-positive, (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, *Micrococcus luteus* ATCC 4698 and *Enterococcus faecalis* ATCC 29212), Gram-negative (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 6539, *Mycobacterium tuberculosis* RCMB 010126) and local strain (*Vibrio cholera*). In addition to some local clinical multi-drug resistant bacteria, *Staphylococcus aureus* (MRSA), *Staphylococcus epidermis*,

Escherichia coli, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumonia.* All tested strains were identified by Vitek® 2 system (bioMarieux, Marcy-LEtoile, France). The multi-drug resistant to antibiotics, namely Ampicillin, Cephalexin, Cefapirin, Cefotaxime, Cefoxitin, Ceftazidime, Colisin, Pepracilline-Tazopactam, Gentamycin, Ipemenem, and Meropenem was verified. Also, it were used to determine the antibacterial activity of the actinomycete isolates. *Fusarium oxysporum* (RCMB 008002), *Aspergillus niger* (RCMB 002007), *Aspergillus flavus* (ATCC 16883), *Penicillium citrinum* (RCMB 001011) and *Candida albicans* (ATCC 10231) were used to define the antifungal activity of the actinomycete isolates.

Preliminary antimicrobial activity:

Each isolate was separately cultivated on the basal salt starch nitrate broth medium [15], set to pH 7.0. The medium included the following components (g/l): 2.0, KNO_3; 20.0, starch; 0.5, $MgSO_4 \cdot 7H_2O$; 1.0, K_2HPO_4; 0.5, NaCl; 3.0, $CaCO_3$ and 0.01, $FeSO_4 \cdot 7H_2O$.

Erlenmeyer flasks (250 ml) including 50 ml of the liquid basal salts medium [16], were inoculating by a disk of 0.4 cm diameter obtained from seven days old culture plates of the examined isolate. The flasks were incubated on a rotary shaker (200 rpm) at 37°C and initial pH 7.0 for four days. The culture broth was centrifuged (6000 rpm at 4°C) to separate the microbial cells. The supernatant was applied to test the antibacterial and antifungal capacity. The antimicrobial ability was expressed as the diameter of the inhibition zones according to the agar plate diffusion method **[17].**

Taxonomic characterizations of actinomycetes isolate, Askar-SH50

Conventional taxonomy:

The characterizations of actinomycete isolate SH50 followed by guidelines adopted with International Streptomyces Project **[**18]. The cultural characteristics were examined according to the guidelines established by the ISP, and colors were evaluated on the scale adopted by Kornerup and Wanscher [19]. Micromorphological studies were carried out by using light and scanning electron microscope (JEOL JSM 5300, JEOL Technics Ltd., Japan) [20,21]. Diaminopimelic acid isomers within this cell-wall also, full cell sugar model were examined employing the purpose of [22]. The biochemical and physiological characteristics; melanin utilization of nitrogen and carbon beginnings, dye generation, enzymatic actions and other physiological characters were also studied [18, 23, 24].

Molecular and phylogenetic identification:

The sequence of a nucleotide for partial 16S rRNA gene of the local isolating actinomycete strain SH50 was done through inoculation of SH50 spores on 50 ml of starch nitrate broth and incubating at 28 °C on a rotary shaker 200 rpm for 72 hours. The total genomic DNA was extracted according to the method of [25]. The 16S rRNA of the strain was increased by PCR employing a GeneAMP PCR System 9700 from PE Applied Biosystems (Perkin Elmer, Ohio, USA). The following primers were employed: F27, 5'-AGAGTTTGATCMTGGCTCAG-3'and R1492 5'-TACGGYTACCTTGTTACGACTT-3and Biolegio BV software (Biolegio, Nijmegen, Netherlands) **[26].** The Amplified products were examined by electrophoresis in 0.7% (w/v) agarose gel and purified using DNA extraction kit (RBC, Korea). The 16S rDNA sequencing was done by ABI PRISM 377 DNA sequencer and ABI PRISM Big Dye Terminator Cycle Sequencing (Perkin Elmer, Ohio, U.S.A) at a sequencing facility at Cornell University in the USA. BLAST (www.ncbi.nlm.gov) obtained to evaluate the DNA similarities. A multiple sequence alignment and molecular phylogenetic analyses were performed using Bio Edit software [27]. The phylogenetic tree was constructed using the Tree View program [28].

Fermentation

Two-disc ($1\text{-}3\times10^6$ spores/disc) from agar culture of the *Streptomyces atroverins*, strain Askar-SH50 (7days old cultures) were applied to inoculate 250 ml Erlenmeyer flasks containing 100ml of the sporulation medium (ISP-3), the initial pH was adjusted at 7.0 before sterilization. The seed flasks were incubated at 36°C for four days on a rotary shaker of 200 rpm. One percent of the vegetative inoculum was then used to inoculate the fermentation flasks, each containing 120 ml of the optimum production medium. The primary pH value of the medium was adjusted at (10.0), before sterilization. After inoculation, the flasks were fixed to a rotary shaker of 180 rpm, and the fermentation was carried out at 40°C. Samples were obtained daily for the assessment of the active substance productivity. At the end of the incubation period, twenty-five liters total volume was filtered through Whatman No.1 filter paper. Fermented culture was centrifuged at 8000 r.p.m (10°C) for 20 minutes to separate the cells.

Extraction

The clear filtrate was adjusted at different pH to select the best pH for the extraction process. The filtrate was subsequently extracted with butanol: acetic acid: water (1:1:8, v/v). The organic form was concentrated to

dryness under vacuum using a rotary evaporator at a temperature 50°C; the residue was dissolved in water and stored at 0-4˚C.

Precipitation
The precipitation process of the crude extract was carried out using petroleum ether (b.p 60-80°C) followed by centrifugation at 6000 rpm (10°C) for 15 min. The supernatant was discarded, and the crude substance was redissolved in the least amount of pure acetic acid and reprecipitated by petroleum ether.

Purification
The purification of the antimicrobial material was carried out using silica gel column (2.5 X 50) chromatography and 0.1N methanolic hydrochloric acid [29], that was used as an eluting solvent. Fifty fractions were collected (each of 5 ml) and tested for their antimicrobial activities **[15].** The active fractions were investigated for its purity by thin layer chromatography (TLC) and using acetic acid (15%) as a solvent system. The active spot was scratched, then dissolved at least amount of water. Finally, the active compound pooled, dried under vacuum to yield a dull white powder and stored at 0-4˚C.

Physico-chemical properties of antimicrobial agent
Elemental analysis:
The elemental analysis (C, H, O, N and S ratio) was carried out at the micro-analytical Center, Cairo University, Egypt.

Spectroscopic analysis:
FT-IR spectrum measurement, it was a useful technique that obtains information about chemical functional groups present in purified antibiotic. The measurements were carrying out using a JASCO FT-IR 3600 infra-red spectrometer by employing KBr Pellet technique and were read at a resolution of 4 cm^{-1} in a wave number region of 400–4000 cm−1. Additionally, purified antibiotic was dissolved in sterile, cold distilled water and was measured using UV–Visible spectrophotometer (T60 UV/Vis. spectrophotometer from 200 to 900 nm at a resolution of 1 nm). On the other hand, Mass spectrum (Thermo scientific single kwad rupole mass spectroscope (ISQLT) was performed to determine molecular weight of purified antibiotic. Also, HNMR and ^{13}C NMR (Bruker Avance (III) 400 MHz, Switzerland) The HNMR and ^{13}C NMR spectra consist of signals that, are assigned to the proton and carbon atoms in purified antibiotic. Surface morphological, and particle size examinations of the purified antibiotic were carried out using Scanning Electron Microscope (SEM) ZEISS, EVO-MA10. Finally, Energy-Dispersive X-ray spectra (EDX) BRUKER Nano GmbH D-12489, 410-M (Berlin-Germany) was used to examine the elemental composition purified antibiotic.

Color tests for antimicrobial agent:
For this purpose, the following reactions were carried out: Molish's, Fehling, Sakaguchi, ninhydrin, Nitroprusside, Ferric chloride and biuret test [30].

Characterization of the antimicrobial agent:
The antimicrobial agent produced by *Streptomyces atroverins*, strains Askar-SH50, was identified according to the recommended international references of [31-34].

Minimum Inhibitory Concentration (MIC) of the purified active compound:
The minimal inhibitory concentration (MIC) of the purified streptomycin was determining by the conventional paper disk diffusion method [35], by applying paper disk (266812 W. Germany 12.7 mm in diameters). Bacteria were grown on nutrient agar medium, while fungi and yeast were grown on Sabouraud agar medium. The purified streptomycin was dissolved in water and loaded on paper disks with different concentrations as the following (250, 125, 62.50, 31.25, 15.63, 7.81, 3.90, 1.95, 0.98, 0.49, 0.24 and 0.12 μg/mL). Drying disks were loaded on surface of agar plates inoculated with test organism. Growth inhibition was examined after 24 hr. from incubation at 37°C for bacteria and after 72 hr. incubation at 27°C for fungi and yeast. Each test was repeated three times. MIC was expressed as the lowest concentration inhibiting test organism's growth.

Anti-mycobacterial activity
Mycobacterium tuberculosis (RCMB 010126) strain was afforded from the culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The separate *M. tuberculosis* (RCMB 010126) clone was grown following anxiety on LB medium at 37 °C for 72 h. The anti-tuberculosis activity was predicted by estimating the diameter of the inhibitory zone, using paper disk diffusion method and measurement of MIC using serial dilution technique. The zones of inhibition were analyzed after 72 h of incubation at 37°C. Each test was repeated 3 three times.

Molecular Docking of streptomycin produced from Streptomyces atroverins, strain Askar-SH50 against Mycobacterium tuberculosis

The molecular modeling study of streptomycin produced from *Streptomyces atroverins*, strain Askar-SH50 was done on an Intel (R). Core(TM) i7-3537U CPU 2.50 GHz, 8.00 GB memory with Windows ten, by operating system using MOE (Molecular Operating Environment) software 2007.09, which provided by chemical computing group, Canada. A systematic conformational search was carried out to an RMS gradient of 0.05Å. Energy minimization of the resultant conformations employing the Conf Search module implemented in MOE. All molecular mechanics computations were conducted by the Merck Force Field (MMFF94s). The crystallographic structure of Mycobacterium tuberculosis enoyl reductase InhA in complex with N-{1-[(2-chloro-6-fluorophenyl) methyl]-1H- pyrazol-3-yl]-5-[(1S)-1-(3-methyl-1H-pyrazol- 1-yl) ethyl]-1,3,4-thiadiazol-2-amine (GSK 625) was obtained from the Protein Data Bank (PDB ID: 5JFO) [36]. Hydrogen atoms were added to the enzyme and partial charges were determined. Validation followed by docking of streptomycin into the active site was carried out, after removing the co-crystallized ligand. The target protein was administered rigid, while the new legand adopts 100 separate confirmations. The best conformer was used based on its S score, and appropriate fitting with the important amino acids in the binding pocket.

Statistical analysis

The means of three replications and standard deviation (SD±) were calculated for all the results obtained, and the data were subjecting to an analysis of variance. Means in the same column with different superscripts are significantly different at ($P< 0.05$) [37].

RESULTS AND DISCUSSION

***In vitro* antibacterial activity**

Eleven actinomycete isolates differ in their cultural, morphological and biochemical characters were isolated in pure form from Sharm El-Sheikh, South Sinai governorate, Egypt, and tested for ability to produce active agent against four different microorganisms (Table 1). From results obtained high percentage (82%) of inhibition was recorded against Gram-positive bacteria than Gram-negative bacteria were less inhibited (41%). *Streptomyces atroverins*, strain Askar-SH50 metabolite, showed antibacterial activity against all the tested microorganisms. The actinomycete isolate, Askar-SH50 was isolated from water sample that collected from South Sinai Sharm El-Sheikh, Egypt. The isolate was growing on starch nitrate agar medium for investigating its potency to produce antimicrobial agents. Studies of antibacterial activity revealed that majority of actinomycete isolates were active against Gram-positive more than Gram negative bacteria [38-41].

***In vitro* antifungal activity**

The antifungal activities of eleven different actinomycete isolates were tested against four different fungi (Table 2). Antifungal activity showed that the degree of antifungal activity varied significantly against among of the fungal pathogens. From antifungal results, 70% of actinomycete isolates were active against one or more of tested fungi. Several researchers have already reported similar antifungal activity of actinomycetes against pathogenic fungi. Augustine and coresearchers (2004) [42] reported that 230 isolates from 335 actinomycetes isolates were active against bacteria, fungi, and yeast. Also, Jain K and Jain C (2004) [43] isolated 287 isolates from various habitats and recorded 166, 164, 134, and 132 actinomycetes isolate active against *C. albicans, A. niger, A. flavus* and *F. oxysporum* respectively.

Table 1: Antibacterial activity of the actinomycetes isolates

Isolates	Mean diameter of inhibition zone (mm)			
	B. subtilis	***S. aureus***	***P. aeruginosa***	***E. Coli***
	ATCC 6633	***ATCC 29213***	**ATCC 27853**	**ATCC 25922**
SSH43	13.0^{c}±0.50	12.0^{c}±0.45	0.0^{a}±0.0	11.0^{c}±0.51
SH 44	16.0^{e}±0.52	20.0^{f}±0.50	21.0^{e}±0.45	0.0^{a}±0.0
SH 45	11.0^{b}±0.45	15.0^{d}±0.52	0.0^{a}±0.005	10.0^{b}±0.50
SH 46	13.0^{d}±0.86	0.0^{a}±0.0	0.0^{a}±0.01	12.0^{d}±0.40
SH 47	0.0^{a}±0.0	11.0^{b}±0.50	0.0^{a}±0.0	0.0^{a}±0.01
SH 48	0.0^{a}±0.005	16.0^{e}±0.49	15.0^{c}±0.50	0.0^{a}±0.005
SH 49	0.0^{a}±0.0	12.0^{bc}±0.45	12.0^{b}±0.40	0.0^{a}±0.0
SH 50	24.0^{f}±0.28	23.0^{h}±0.46	21.0^{e}±0.40	18.0^{e}±0.45
SH 51	14.0^{d}±0.58	16.0^{e}±0.60	0.0^{a}±0.0	0.0^{a}±0.01
SH 52	13.0^{c}±0.58	12.0^{c}±0.46	0.0^{a}±0.01	0.0^{a}±0.0
SH 53	12.0^{b}±0.17	22.0^{g}±0.25	19.0^{d}±0.45	0.0^{a}±0.005
LSD (0.05)	0.88	0.96	1.8	0.66

The results are represented as (Mean ± SD), LSD: Least Significant Difference, mean values were followed by the same letters and column were not significantly different (Duncan's multiple range test) at $p< 0.05$.

Table 2: Antifungal activity of the actinomycetes isolates

Isolates	Mean diameter of inhibition zone (mm)			
	C. albicans	***A. niger***	***A. flavus***	***F. oxysporum***
	ATCC 10231	**RCMB 002007**	***ATCC 16883***	***RCMB 008002***
SH43	0.0 ^{a}±0.15	20.0^{e}±0.015	20.0^{e}±0.015	18.0^{c}±0.28
SH 44	0.0^{a} ±0.01	12.0^{b}±0.01	0.0^{a}±0.1	15.0^{b}±0.46
SH 45	16.0^{d} ±0.45	16.0^{cd}±0.45	15.0^{c}±0.45	25.0^{e}±0.34
SH 46	17.0 ^{e}±0.36	16.0^{cd}±0.36	15.0^{c}±0.36	25.0^{e}±0.40
SH 47	0.0^{a} ±0.01	16.0^{cd}±0.01	17.0^{d}±0.01	23.0^{d}±0.45
SH 48	0.0 ^{a}±0.015	14.0^{bc}±0.015	17.0^{d}±0.15	0.0^{a}±0.01
SH 49	0.0 ^{a}±0.01	14.0^{bc}±0.01	17.0^{d}±0.01	0.0^{a}±0.015
SH 50	21.0 ^{f}±0.45	16.0^{cd}±0.45	14.0^{b}±0.45	15.0^{b}±0.45
SH 51	15.0 ^{c}±0.26	21.0^{de}±0.26	20.0^{e}±0.26	28.0^{f}±0.15
SH 52	13.0 ^{b} ±0.43	16.0^{cd}±0.43	20.0^{e}±0.43	25.0^{e}±0.41
SH 53	0.0 ^{a}±0.01	0.0^{a}±0.01	0.0^{a}±0.01	0.0^{a}±0.01
LSD (0.05)	0.9	0.66	0.96	1.76

The results are represented as (Mean ± SD), LSD: Least Significant Difference, mean values were followed by the same letters and column were not significantly different (Duncan's multiple range test) at $p< 0.05$.

Taxonomic characterizations of actinomycete isolate Askar-SH50

Conventional taxonomy:

The most active actinomycete isolate Askar SH-50 was inoculated on agar media adopted by International Streptomyces Project (ISP). After incubation for 7, 14 and 21days at 37˚C, cultural characteristics of isolate are presenting in the (Table 3). The color of the aerial mycelium appeared very pale green to moderate bluish green, while that of substrate mycelium range from dark grayish green to light yellow.

The strain produced deep green pigments on ISP-3 and exhibited melanin pigmentation on ISP-6 and ISP-7 media. The strain showed superior growth on ISP-4 and ISP-3, great growth on ISP 6, 7 and weak growth on ISP 1, 2 media. Micromorphological characteristics of actinomycete strain, Askar SH-50 grown on inorganic salts-starch agar (ISP-4) under light microscopy and scanning electron microscope (Figure 1 and Figure **2)** exhibited spiral shaped mycelium that further differentiated into hairy surfaced spores, and unique morphological characteristics.

Whole cell hydrolyzate of this strain contained LL-diaminopimelic acid (LL-DAP) chemo-type I cell wall, but no specific sugars could be recognized. Cell-wall composition analysis is one of the primary techniques that can be manipulated to distinguish the chemotaxonomic properties of *Streptomyces*. Identification process has been carried out [38,44**],** and numerical taxonomy of *Streptomyces* species.

For the purpose of identification of actinomycete isolate, the morphological characteristics and microscopic examinations emphasized that the spore chain is spiral. Spore surface is hairy, the color of the aerial mycelium appeared very pale green to moderate bluish green, while, substrate mycelium range from dark grayish green to light yellow. The strain produced deep green pigments on ISP-3 and exhibited melanin pigmentation on ISP-6 and ISP-7 media.

The present results of physiological, biochemical characteristics and cell wall hydrolysate of actinomycetes isolate, exhibited that the sugar pattern of cell wall hydrolysate could not detected and presence of LL--diaminopimelic acid (LL-DAP) in the cell wall. Also these results signified that the actinomycete isolate related to a group of *Streptomyces* [44,45].

The physiological and biochemical properties include on, carbon and nitrogen sources utilization, enzymatic activities, tolerance to NaCl, growth at different pH, growth on different temperature, growth inhibitors and resistance to antibiotics were present in (Table 3). The isolate under investigation was found to produce the following enzymes (amylase, protease, catalase and nitrate reductase) and utilized D-galactose, D-glucose, D-xylose, L-arabinose, maltose, lactose, mannitol, raffinose, cellulose, starch and sucrose as carbon sources that indicating its broad pattern of carbon assimilation .

It showed sensitivity to a variety of antibiotics but was resistant to Penicillin, and norfloxacin. Kampfer and coresearchers (1991) [24], was suggested that all these tests are indispensable tools for classification of actinobacteria and suggesting that bioactive compounds produced by the strain may be responsible for the resistance of the strain to the antibiotics.

Table 3: Cultural characteristics of actinomycete Askar SH-50 grown on different ISP media

Culture media	Growth	Age (days)	Color of substrate mycelium	Color of aerial mycelium	Color of diffusible pigments
Tryptone yeast extract broth (ISP–1)	Weak	7	White (ISCC-NBS 263)	White (ISCC-NBS 263)	strong reddish brown (ISCC-NBS 40)
		14	light gray (ISCC- NBS 264)	White (ISCC-NBS 263)	strong reddish brown (ISCC-NBS 40)
		21	light gray (ISCC- NBS 264)	White (ISCC-NBS 263)	strong reddish brown (ISCC-NBS 40)
Yeast -malt extract agar (ISP–2)	Weak	7	White (ISCC-NBS 263)	White (ISCC-NBS 263)	vivid greenish yellow (ISCC- NBS 97)
		14	light yellow (ISCC-NBS 86)	light gray (ISCC- NBS 264)	vivid greenish yellow (ISCC- NBS 97)
		21	light yellow (ISCC- NBS 86)	light gray (ISCC- NBS 264)	vivid greenish yellow (ISCC- NBS 97)
Oatmeal agar (ISP–3)	Excellent	7	light gray (ISCC- NBS 264)	light gray (ISCC- NBS 264)	deep green (ISCC- NBS 142)
		14	dark grayish green (ISCC- NBS 151)	dark gray (ISCC- NBS 266)	deep green (ISCC- NBS 142)
		21	dark grayish green (ISCC- NBS 151)	dark gray (ISCC- NBS 266)	deep green (ISCC- NBS 142)
Inorganic-trace salt- starch agar (ISP–4)	Excellent	7	White (ISCC-NBS 263)	White (ISCC-NBS 263)	None
		14	light yellow (ISCC-NBS 86)	light gray (ISCC- NBS 264)	None
		21	light yellow (ISCC-NBS 86)	light gray (ISCC- NBS 264)	None
Glycerol asparagine agar (ISP–5)	Weak	7	moderate reddish brown (ISCC-NBS 43)	White (ISCC- NBS 263)	moderate yellowish brown (ISCC- NBS 77)
		14	deep reddish brown (ISCC-NBS 41)	White (ISCC- NBS 263)	moderate yellowish brown (ISCC- NBS 77)
		21	deep reddish brown (ISCC-NBS 41)	White (ISCC- NBS 263)	moderate yellowish brown (ISCC- NBS 77)
Peptone yeast extract iron agar (ISP–6)	Good	7	light yellowish brown (ISCC- NBS 76)	White (ISCC- NBS 263)	Dark yellowish brown (ISCC-NBS 78)
		14	deep yellowish brown (ISCC- NBS 75)	White (ISCC- NBS 263)	Dark yellowish brown (ISCC-NBS 78)
		21	deep yellowish brown (ISCC- NBS 75)	White (ISCC- NBS 263)	Dark yellowish brown (ISCC-NBS 78)
Tyrosine agar (ISP–7)	Good	7	deep brown (ISCC- NBS 56)	yellowish white (ISCC- NBS 92)	strong yellowish brown (ISCC-NBS 74)
		14	Black (ISCC- NBS 267)	dark grayish reddish brown (ISCC- NBS 47)	strong yellowish brown (ISCC-NBS 74)
		21	Black (ISCC- NBS 267)	dark grayish reddish brown (ISCC- NBS 47)	strong yellowish brown (ISCC-NBS 74)

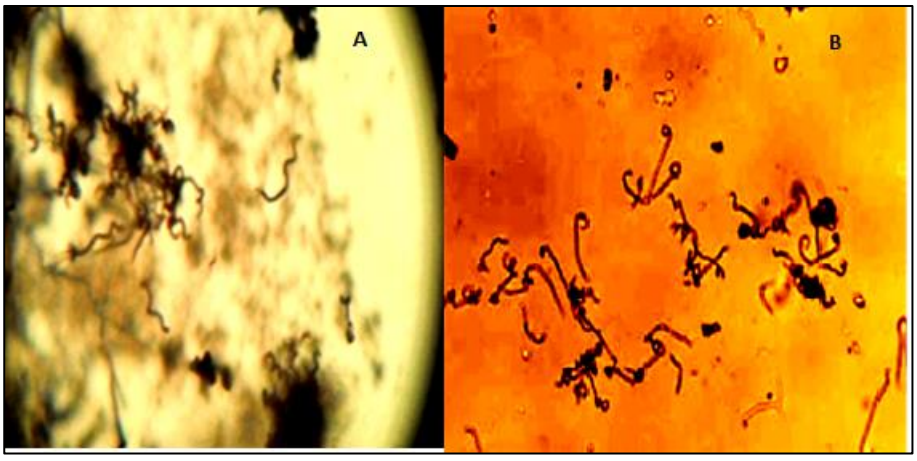


Figure 1: Phase-contrast micrograph of actinomycete strain, Askar SH-50 showing spiral shaped mycelium

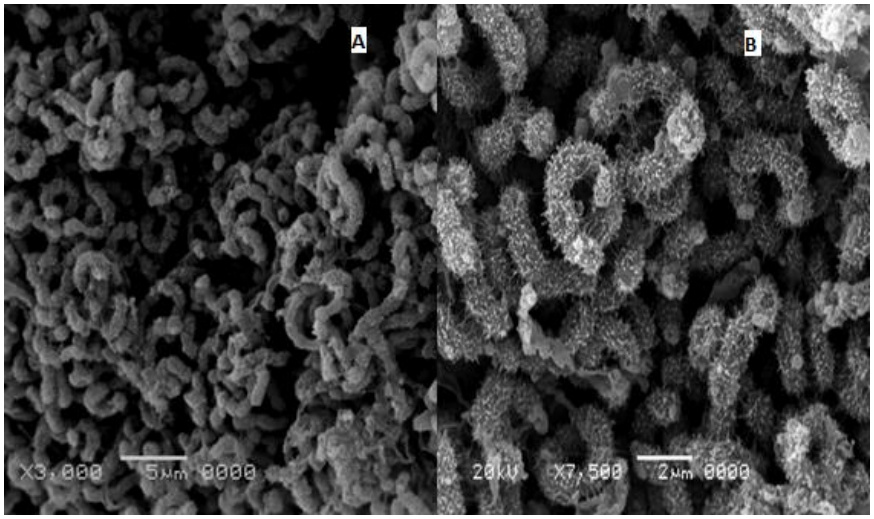


Figure 2: Scanning electron microscopy (SEM) showing (A) spiral shaped mycelium that further differentiated into hairy surfaced spores (B)

16S rRNA gene sequencing and phylogenetic analysis:

The16S rRNA gene sequence of the local isolate was comparing to sequences of 15 *Streptomyces* sp. to confirm identification of the actinomycete isolate, Askar SH-50, The results obtained from PCR amplification and agarose gel electrophoresis exhibited specific 16S rRNA band at 1000bps (Figure 3).The phylogenetic tree (Figure 4) showed that the locally isolated strain is closely related to *Streptomyces atroverins*, that was constructed using the neighbour-joining method with the aid of genius pro 7.1.5 tree builder program. Bar 0.20 substitutions per nucleotide position. Multiple sequence alignment was done between the sequences of the 16S rRNA genes of *Streptomyces atroverins* and other eight *Streptomyces* sp. and the local isolate. Computer-assisted DNA similarly searches against bacterial database revealed that 16S rRNA sequence was 99% identical to *Streptomyces atroverins*, and depositing in NCBI gene bank as *Streptomyces atroverins*, strains Askar SH-50, under accession number KU740212.

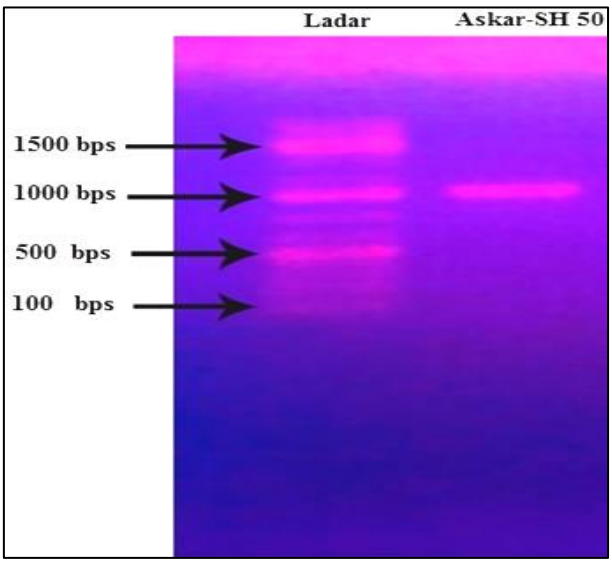


Figure 3: Gel electrophoresis for PCR product of DNA extracted from strain Askar SH-50

Table 4: Physiological and biochemical characteristics of actinomycetes strain, Askar SH-50

Character	Results
Melanin pigment	
Tryptone-yeast extract broth	+
Peptone-yeast extract iron agar	+
Tyrosine agar	+
Enzymatic activities	
Amylase	++
Protease	+
Lipase	–
Pectinase	–
Catalase	+
Gelatinase	+
Nitrate reductase	+
Urease	–
Xanthine degradation	+
H2S production	+
Carbon sources utilization	
D–arabinose	Wg[e]
L–arabinose	Wg[e]
D–xylose	Wg[e]
L–xylose	Wg[e]
D–galactose	++
D–glucose	+++[a]
D–fructose	++
Maltose	++
Rhamnose	+++[a]
Sucrose	++
Mannitol	+++[a]
Starch	+++[a]
Cellulose	+
Esculin	-
Nitrogen sources utilization	
L-histidine	Wg[e]
L-threonine	+
L-serine	+
L-arginine	+
L-cysteine	Wg[e]
Tyrosine	+++[a]
Peptone	+++[a]
Yeast extract	+++[a]
Urea	++
Ammonium sulfate	+
Tolerance to NaCl concentrations	
1.0 - 6.0	++
7	+
8.0–14	–
Growth temperature ^{o}C	
10	–
20	+
30	+
40.0 - 45.0	++
50.0 - 60.0	–
Growth pH	
3.0 - 8.0	++
9.0 - 12.0	++
13.0 - 15.00	–
Tolerance to growth inhibitors	
Sodium azide (0.01%)	+++
Sodium azide (0.02%)	+++
Phenol (0.1%)	–
Crystal violet (0.0001%)	+++
Thallus acetate (0.001%)	–
Resistance to antibiotics	
Erythromycin(15µgm)	++
Penicillin (25 µg/ml)	-
Ciprofloxacin(30µgm)	++
Tetracycline (15µgm)	+++
Bacitracin (50 µgm)	-
Norfloxacin (30 µgm)	-
Rifampicin (50µg/ml)	-
Chloramphenicol(30µgm)	++

[a](+++) = abundant growth, [b](-) = negative, [c](++) = good growth, [d](+) = moderate, [g](NG) = No growth.[e](wg) = Weak growth.

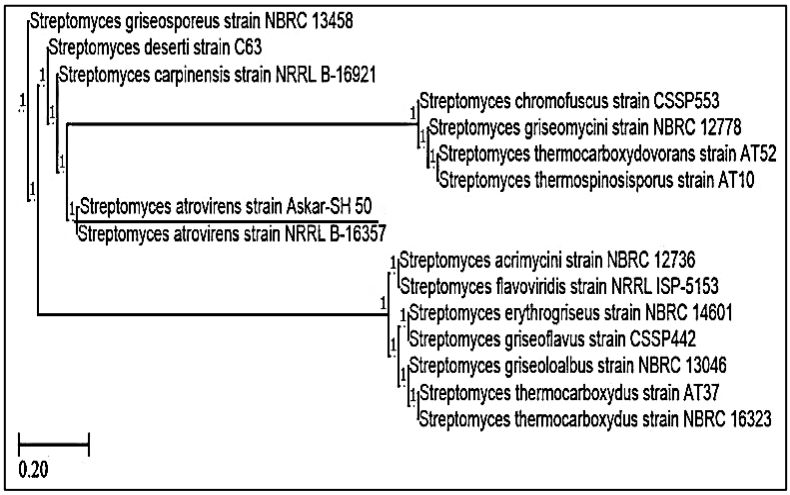


Figure 4: The phylogenetic tree of *Streptomyces atroverins*, strains Askar SH-50

Fermentation, extraction and purification of the active metabolite

The fermentation process was carried out for five days at 40°C using liquid starch nitrate as production medium. Twenty-five liter total volume filtrate was conducted followed by centrifugation at 5000 rpm. at 10°C for 20 minutes. The clear filtrates containing the active metabolite (24 liters), was adjusted to pH 7.0, then extraction process was carried out using butanol: acetic acid: water (1:1:8, v/v). The organic phase was collected and evaporated under reduced pressure using a rotary evaporator. The antimicrobial compound was precipitated by petroleum ether (b.p. 60-80°C) and centrifuged at 6000 RPM at 10°C for 15 minutes. The purification process through column chromatography packed with silica gel and an eluting solvent composed of methanolic hydrochloric acid (0.1N), revealed that the most active fractions from No.23 to No. 30. Confirmation from the purification of antimicrobial agent into individual components was carried out by thin-layer chromatography using a solvent system composed of acetic acid (15%) Only one band at Rf = 0.75 showed antimicrobial activity (Figure 5 and Figure 6). The purification process through column chromatography packed with silica gel and an eluting solvent composed of methanolic hydrochloric acid (0.1N), revealed that the most active fractions from 23 to 30 [29].

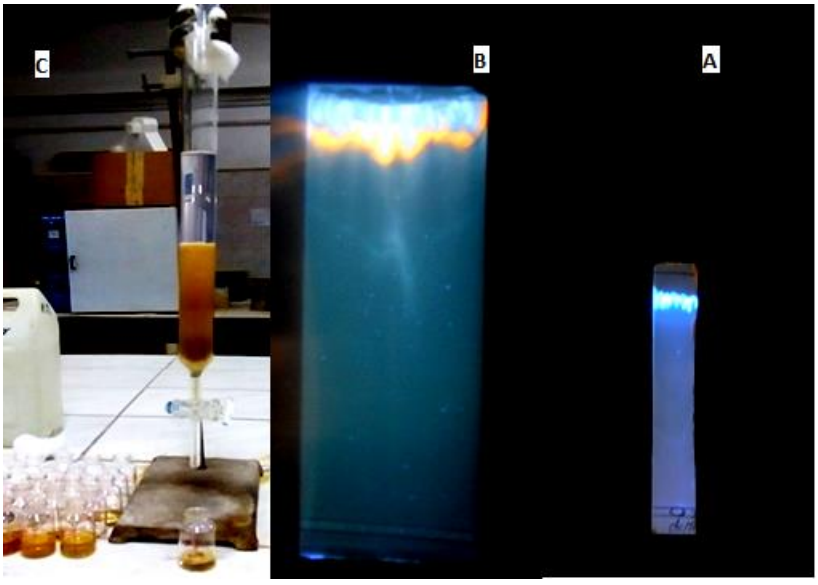


Figure 5: Purification process was carried out by (A) column chromatography and (B) Thin layer chromatography using acetic acid (15%); while (C) Active spot under UV

Figure 6: Bio-autography for purified active compound on thin layer chromatography against Bacillus subtilis

Characterization of purified antimicrobial agent

Physicochemical Characteristics:

The purified antimicrobial agent is soluble in water, acetone, acetic acid, and carbon tetrachloride but, insoluble in DMSO, methanol, chloroform, and benzene, also poorly soluble in n-butanol, hexane and with a melting point of range from 205°C to 210°C.

Elemental analysis:

The elemental analytical data of the antimicrobial agent revealed the following data: C= 43.37 %; H= 6.76%; N= 16.86% and O = 33.01%. From the analysis, data suggested calculated empirical formula $C_{21}H_{39}N_7O_{12}$.

Biochemical reactions of the antimicrobial agent:

The color reactions of the purified antimicrobial agent to detect certain groups were investigated. The antimicrobial agent exhibited positive results with molish's, sakaguchi and nitroprusside while giving negative results with ninhydrin, feeling, ferric chloride and biuret tests.

Spectroscopic characteristics:

The ultraviolet (UV) absorption spectrum of the antimicrobial agent recorded a maximum absorption peak at 210 nm. (Figure 7). The infrared (IR) spectrum of the antimicrobial agent as showed in (figure 8). The Mass spectrum exhibited that the molecular weight is 581.57 (Figure 9). The HNMR and ^{13}CNMR analysis of antimicrobial agent showed in (figure 10 and figure 11).

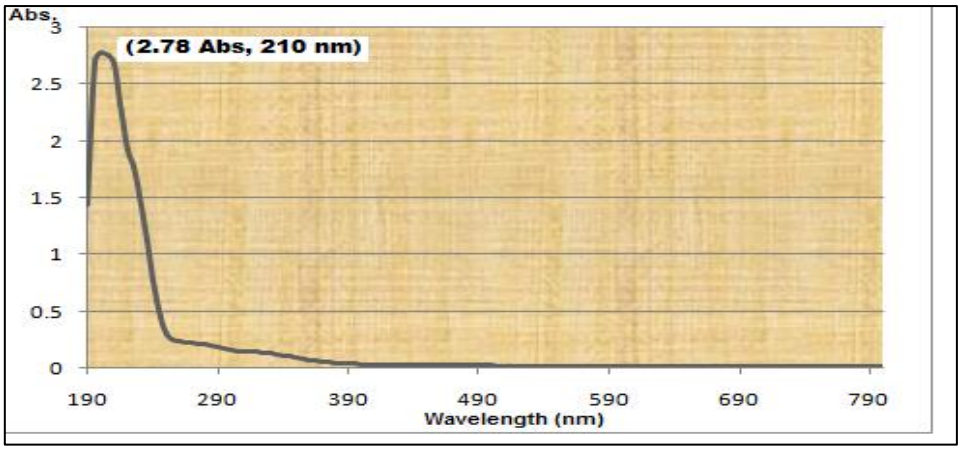


Figure 7: Ultraviolet absorbance of the purified

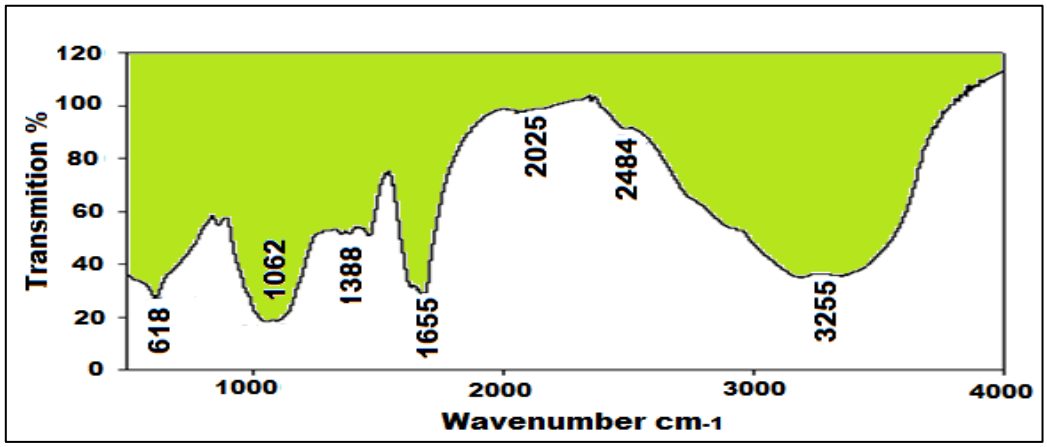


Figure 8: Infrared spectrum of the purified antimicrobial agent

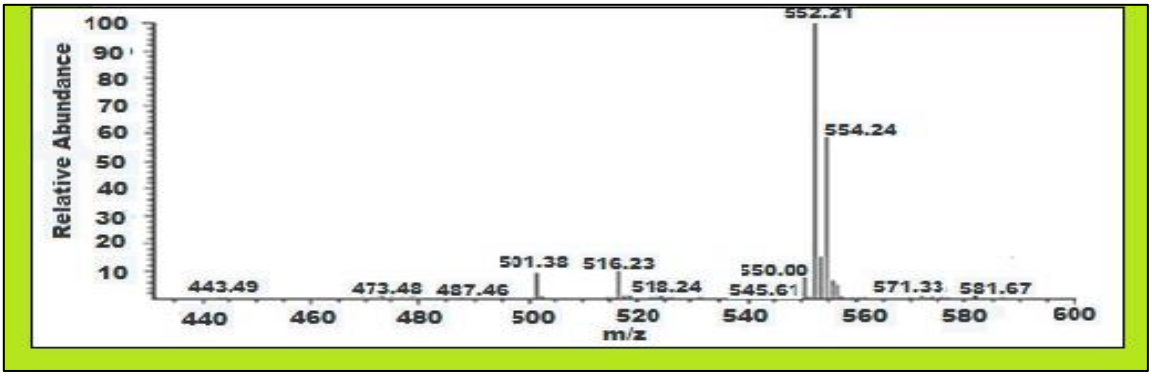


Figure 9: Mass Spectrum of the purified antimicrobial agent

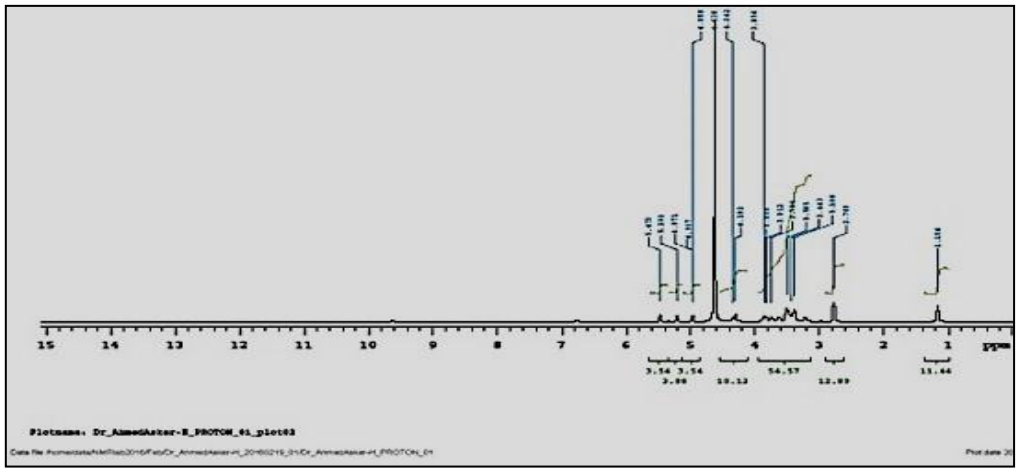


Figure 10: HNMR of the purified antimicrobicrbial agent

According to physicochemical characteristics, elemental analysis, biochemical reactions and spectroscopic characteristics, the expected structure of antimicrobial agent is streptomycin. The interpretation of ^{13}C NMR supported this expectation as showing in table (5). Comparative study between streptomycin produce by *Streptomyces atroverins* strain Askar-SH50 and reference standard streptomycin showing in table (6).

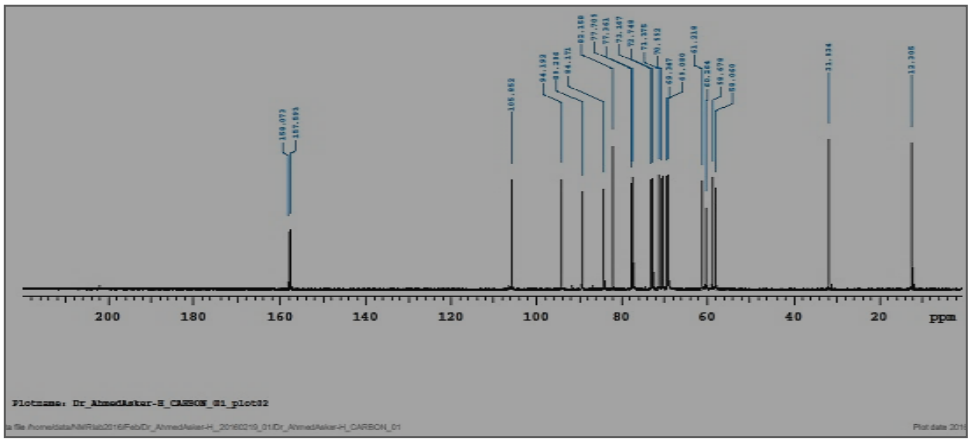


Figure 11: ^{13}C NMR of the purified antimicrobial agent

Table 5: Chemical Shifts of the 13C NMR of the produced Streptomycin

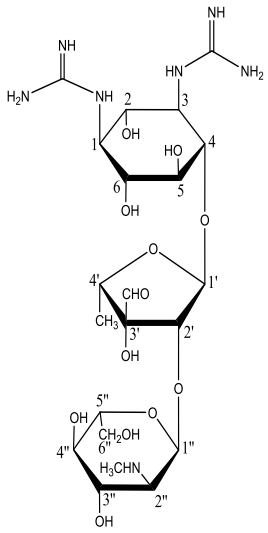
Chemical Shifts of the ^{13}C NMR of Streptomycin		
Streptomycin	**No of C**	**location**
	1	58.678
	2	70.552
	3	58.06
	4	77.705
	5	73.167
	6	71.375
	1'	105.852
	2'	77.361
	3'	82.158
	4'	84.171
	1"	94.192
	2"	61.218
	3"	69.08
	4"	69.347
	5"	72.748
	6"	60.264
	C-CH_3	12.305
	N-CH_3	31.934
	H-C=O	89.192
	C=NH (C1)	158.073
	C=NH (C3)	157.593

Table 6: A comparative study between streptomycin produce by Streptomyces atroverins strain Askar-SH50 and reference standard streptomycin

Characteristic	**Reference standard streptomycin**	**Purified streptomycin produced by *Streptomyces atroverins* strain Askar-SH50**
Melting point	200-205°C	200-205°C
Molecular weight	581.57 g/mol	581.57 g/mol
C%	43.37	43.37
H%	6.76	6.76
N%	16.86	16.86
O%	33.01	33.01
Ultra violet-Abso.	200 nm	210 nm
Formula	$C_{21}H_{39}N_7O_{12}$	$C_{21}H_{39}N_7O_{12}$

The spectroscopic characteristics of the antimicrobial agent under study revealed the ultraviolet (UV) absorption spectrum recorded a maximum absorption peak at 200 nm. The Mass spectrum revealed that the molecular weight 581.57 [29]. The ^{13}C spectrum confirmed the structure of streptomycin. The spectra consist of signals are assigned to the two carbon atoms of the guanidine groups, δ 158.073, 157.593 ppm. Signals appear at 58.678, 58.060 assigned to the two carbon atoms no C-1, C- 3 bearing the guanidine substituents. Also, signals appear at 70.552, 77.705 assigned to the two carbon atoms no C-2, C-5. The strong-field one is assigned to the C-2 carbon

having the voluminous guanidine substituent on the C-I and C-3 carbon atoms and the weak-field one to C-5. Signals appear at 77.705, 71.375 assigned to the C-4 and C-6 carbon atoms bearing hydroxy groups. The substitution of the streptidine hydroxyl at C-4 by the streptobiosaminyl substituent should lead to more considerable changes in the CS values of these carbon atoms and of those closest to it, C-3 and C-5, and also of the carbon atom of the guanidine group at C-3. The signal of the C-2' carbon atom of the spectrum of methyl streptobiosaminide appears at 77.361 closer to C-4 that appear at 77.705. While, signals appear at 105.852 is assigned to C-1` closer to C-1`` appear at 94.192. The C-5" appear closer to C-5 they appear at 72.748 and 73.167, respectively [33]. The biochemical tests of antimicrobial agent exhibited positive results with molish's, sakaguchi and nitroprusside while giving negative results with ninhydrin, feeling, ferric chloride and biuret test [29]. Identification of antimicrobial agent according to recommended international keys indicated that the antibiotic is suggestive of being belonging to Streptomycin antibiotic [31, 32, 33, 34, 29]. Aminoglycoside is registering in the treatment of serious systemic infections for which less toxic antibacterial are worthless or contraindicated. The spectrum of aminoglycosides includes aerobic Gram-negative bacilli and some Gram-positive organisms. They are not active against anaerobic organisms [47]. On the other hand, SEM results founded in Figure 12, showing the surface morphology of the purified antibiotic with circular shape with different sizes. While, in Figure 13, EDX analysis of the purified antibiotic explained the elemental analysis of it is components and was founded that, the purified antibiotic contained carbon, nitrogen, and oxygen. On the other hand, Sulfur was traced from the media component during the fermentation process of antibiotic production.

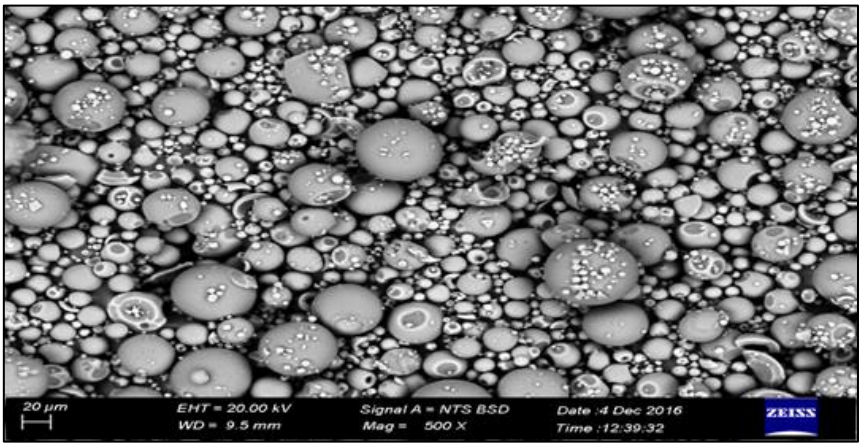


Figure 12: Characterization of the purified antibiotic using Scanning Electron Microscope

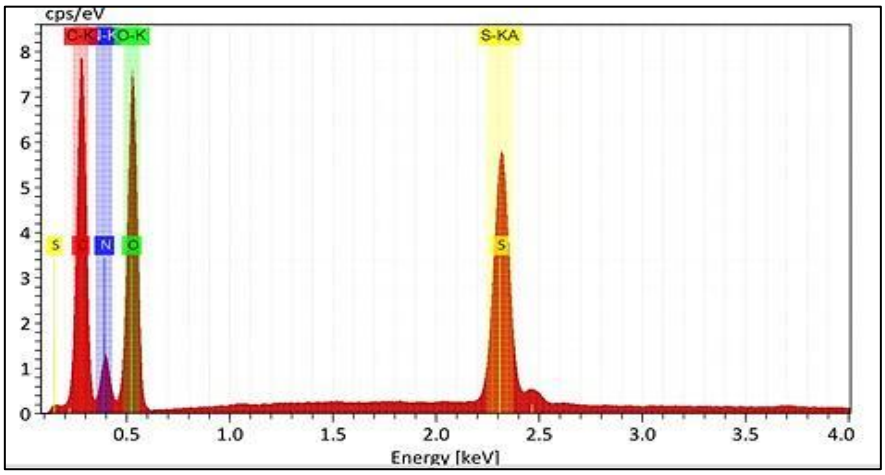


Figure 13: Characterization of the purified antibiotic using EDX analysis

Antimicrobial activity of streptomycin produced by *Streptomyces atroverins,* strain Askar- SH50

***In vitro* Antibacterial activity:**

The antibacterial activity of streptomycin produced by *Streptomyces atroverins*, strain Askar- SH50 exhibited antibacterial activity against both Gram- positive and Gram-negative bacteria as shown in the Table (7). The zone of inhibition (ZOI) ranging from 23.0 mm in *Staphylococcus aureus* (MRSA) to 70.0 mm in *Micrococcus luteus*, also active against *Mycobacterium tuberculosis* with a zone of inhibition 30 mm.

The MIC of streptomycin produced by *Streptomyces atroverins*, strain Askar- SH50 was determined, and the results showed that the minimum inhibitory concentration ranging from 0.48µg/ml against *Micrococcus luteus* up to (125µg/ml *Enterococcus faecalis* and *Staphylococcus aureus MRSA*) and 15.62µg/ml against *Mycobacterium tuberculosis*.

In comparison the activity of standard streptomycin antibiotic with purified antibiotic, we noted high similarity in the activity. Where the antibacterial activity of standard streptomycin antibiotic show antibacterial activity against both Gram- positive and Gram-negative bacteria as shown in table (7) The zone of inhibition (ZOI) ranging from 25.0 mm in *Acinetobacter baumannii* (resistance strain) to 65.0 mm in *Micrococcus luteus*, also active against *Mycobacterium tuberculosis* with a zone of inhibition 30mm.

The MIC of standard streptomycin antibiotic was determined, and the results showed that the MIC ranging from 0.97µg/ml against *Micrococcus luteus* to 125µg/ml against *Pseudomonas aeruginosa*, and 15.62µg/ml against *Mycobacterium tuberculosis*.

While the activity of standard tetracycline ranging from 20 mm against *Pseudomonas aeruginosa* to 30 mm *Salmonella typhi*, with MIC ranging from 7.81 µg/ml against *Fusarium oxysporum* and *Candida albicans* to 62.5 µg/ml against *Staphylococcus aureus and Pseudomonas aeruginosa*. Streptomycin is used primarily as an anti-tubercular and is active against *Mycobacterium tuberculosis* and *Mycobacterium bovis*. It is also examined the drug of opportunity for the treatment of infections produced by *Francisella tularensis* and *Yersinia pestis* and is often used to treat *Brucella* infections. Because many other Gram-negative bacilli are resistant, streptomycin is rarely used to deal with those microorganisms [47].

The initial intracellular position of action of the aminoglycosides is the 30S ribosomal subunit, that consists of a single 16S molecule of RNA and 21 proteins. At least three of these proteins and perhaps the 16S ribosomal RNA as well add to the streptomycin binding site, and modifications of these molecules particularly affect the binding and subsequent action of streptomycin. For instance, a particular amino acid replacement of asparagine for lysine at point 42 of one ribosomal protein (S12) prevents binding of the drug; the resultant mutant is entirely resistant to streptomycin.

Table 7: Antibacterial activity of streptomycin produced by Streptomyces atroverins, strain Askar- SH50

Microbial test strains		Mean diameter of inhibition zone (mm)/ minimum inhibitory concentration (MIC) (µg/ml)					
		Streptomycin **produced from *Streptomyces atroverins*, strain Askar- SH50**		***Streptomycin*** **(standard)**		**Tetracycline (standard)**	
		Inhibition zone	**MIC**	**Inhibition zone**	**MIC**	**Inhibition zone**	**MIC**
Bacillus subtilis (ATCC 6633)	Standard strains (ATCC)	33^{e}±0.01	31.29^{f}±0.005	35^{d} ±0.1	31.29^{f}±0.005	25^{b}±0.005	31.25^{d}±0.005
Staphylococcus aureus (ATCC 29213)		30^{d}±0.02	62.5^{g}±0.05	33^{d}±0.1	62.5^{g}±0.17	25^{c}±0.005	62.5^{e}±0.005
Micrococcus luteus (ATCC 4698)		70^{k}±0.01	0.48^{a}±0.005	65^{f}±0.01	0.97^{a}±0.01	22^{d}±0.005	15.62^{c}±0.005
Escherichia coli		35^{h}±0.11	7.81^{d} ±0.005	39^{ef}±0.1	3.90^{c} ±0.005	23^{e}±0.005	15.62^{c}±0.005
Pseudomonas aeruginosa (ATCC 27853)		28^{f}±0.01	31.29^{f}±0.005	30^{abc}±0.1	31.29^{f}±0.01	20^{c}±0.01	62.5^{e}±0.005
Enterococcus faecalis (ATCC 29212)		30^{g}±0.02	125^{h}±0.01	32^{ab}±0.1	62.5^{h}±0.05	0.0^{a}±0.01	0.0^{a}±0.01
Salmonella typhi		40^{h}±0.01	3.90^{c}±0.005	40^{d}±0.05	3.90^{c}±0.01	30^{g}±0.01	7.81^{b}±0.005
Vibrio cholera		45^{l}±0.015	1.95^{b}±0.005	45^{cde}±0.1	1.95^{b}±0.01	24^{f}±0.01	31.25^{d}±0.005
Mycobacterium tuberculosis (RCMB 010126)		30^{h}±0.01	15.62^{e}±0.005	30^{abc}±0.1	15.62^{e}±0.005	0.0^{a}±0.01	0.0^{a}±0.01
Staphylococcus aureus (MRSA)	Clinical resistance strains	23^{a}±0.01	125^{h}±0.01	26^{a}±0.1	62.5^{h}±0.05	0.0^{a}±0.01	0.0^{a}±0.01
Staphylococcus epidermis		47±0.01	15.62^{e}±0.005	47^{df}±0.1	15.62^{e}±0.005	0.0^{a}±0.01	0.0^{a}±0.01
Escherichia coli		40^{i}±0.01	3.90^{c} ±0.005	40^{d}±0.1	3.90^{c} ±0.005	0.0^{a}±0.01	0.0^{a}±0.01
Pseudomonas aeruginosa		30^{c}±0.01	62.5^{g}±0.05	26^{a}±0.01	125^{i}±0.01	0.0^{a}±0.01	0.0^{a}±0.01
Acinetobacter baumannii		25^{b}±0.15	7.81^{d}±0.005	25^{a}±0.1	7.81^{d}±0.005	0.0^{a}±0.01	0.0^{a}±0.01
Klebsiella pneumonia		41^{g}±0.01	3.90^{c}±0.005	43^{cde}±0.15	3.90^{c}±0.005	0.0^{a}±0.01	0.0^{a}±0.01
LSD		0.99	1.47	0.96	0.13	0.006	7.8

The results are represented as (Mean ± SD), LSD: Least Significant Difference, mean values were followed by the same letters and column were not significantly different (Duncan's multiple range test) at $p< 0.05$.

***In vitro*Antifungal activity:**

From results, it was found that, streptomycin produced from *Streptomyces atroverins*, strain Askar- SH50 showed antifungal activity against both unicellular and filamentous fungi as shown in the table (8). The zone of inhibition (ZOI) ranging from 13 mm against *Aspergillus flavus* to 23 mm *Candida albicans*. The MIC of antifungal was determined, and the results showed that the minimum inhibitory concentration (MIC) against

unicellular and multicellular fungi ranging from15.62 µg/ml *Fusarium oxysporum* and *Candida albicans* to 125µg/ml *Aspergillus flavus*.

In addation, compared with antifungal activity of standard streptomycin antibiotic, was noted high similarity in the activity with purified antibiotic. That showed antifungal activity against both unicellular and filamentous fungi, where, ZOI ranging from 10 mm against *Aspergillus flavus* to 23 mm *Candida albicans* as shown in table 5. The MIC of antifungal were determined, and the results showed that MIC against unicellular and multicellular fungi ranging from 15.62µg/ml *Fusarium oxysporum* and *Candida albicans* to 250 µg/ml *Aspergillus flavus*. While the activity of standard amphotericin B ranging from 16 mm against *Aspergillus niger* to 18.0 mm *Fusarium oxysporum*, with MIC ranging from15.62 µg/ml against *Fusarium oxysporum* and *Candida albicans* to 62.5 µg/ml *Aspergillus niger and Penicillium citrinum* as shown in figure (14). Different mutant in that, Glutamine comprises the amino acid at this location, is subordinate toward streptomycin [48].

Further, streptomycin is applied as a pesticide, to fight the majority of bacteria, algae, and, fungi. Streptomycin was checking bacterial and fungal diseases of certain fruit, vegetables, seed, and ornamental crops. [49]. The antifungal protection provided by copper- streptomycin chelate *in vivo* in nursery experiments toward Phytophthora infestans on tomato was observed to be in the form of six times higher than that of streptomycin sulfate [50].

Table 8: Antifungal activity of streptomycin produced by Streptomyces atroverins, strain Askar- SH50

Microbial test strains	Mean diameter of inhibition zone (mm)/ minimum inhibitory concentration (MIC) (µg/ml)					
	Streptomycin produced from *Streptomyces atroverins*, strain Askar- SH50		*Streptomycin* (standard)		Amphotericin B (standard)	
	Inhibition zone	MIC	Inhibition zone	MIC	Inhibition zone	MIC
Candida albicans ATCC 10231	23^{e} ± 0.23	15.62^{a} ±0.005	23^{e}±0.05	15.62^{a} ±0.005	0.0^{a}±0.01	0.0^{a} ±0.01
Aspergillus niger (RCMB 002007)	15^{b}± 0.29	31.25^{b} ±0.01	13^{b} ±0.05	31.25^{b} ±0.005	16^{b}±0.15	62.5^{d}±0.05
Aspergillus flavus (ATCC 16883)	13^{a}± 0.15	125^{c} ±0.01	10^{a} ±0.11	250^{c} ±0.05	17^{c}±0.12	31.25^{c}±0.01
Fusarium oxysporum (RCMB 008002)	20^{d} ± 0.25	15.62^{a} ±0.005	20^{d}±0.05	15.62^{a} ±0.005	18^{d}±0.17	15.62^{b}±0.005
Penicillium citrinum (RCMB *001011*)	17^{c} ± 0.26	31.9^{b}±0.005	15^{c}±0.11	31.9^{a}±0.005	17^{c}±0.20	62.5^{d}±0.05
LCD	1.97	15.66	0.33	11.53	0.8	15.61

The results are represented as (Mean ± SD), LSD: Least Significant Difference, mean values were followed by the same letters and column were not significantly different (Duncan's multiple range test) at p< 0.05

Molecular Docking of streptomycin produced from *Streptomyces atroverins*, strain Askar-SH50 against *Mycobacterium tuberculosis.*

Molecular modeling was performed to determine the possible binding interactions of streptomycin produced from *Streptomyces atroverins*, strain Askar-SH50 in the active site of *Mycobacterium tuberculosis* enoyl-reductase InhA and to predict its mode of action. The protein data bank file (PDB ID: 5JFO) was selected for this purpose. The file contains Mycobacterium tuberculosis enoyl-reductase InhA enzyme co-crystallized with the legand N-[1-[(2-chloro-6-fluorophenyl) methyl]-1H-pyrazol-3-yl]-5-[(1S)-1-(3-methyl-1H-pyrazol-1-yl) ethyl]-1,3,4 thiadiazol-2-amine (GSK 625). The Docking study was validated through the re-docking of the co-crystallized legand inside the active site of the enzyme with an energy score (S) = -10.66 Kcal mol-1. Then streptomycin produced from *Streptomyces atroverins*; strain Askar-SH50 was fit in the active site with a score (S) = -12.57 Kcal mol-1.

The 2D interactions of a legand with the amino acids of the active site are shown figure (15). To visualize these interactions in better manner 3D interactions was illustrated in figure (16). Validation of the molecular docking protocol indicates that, streptomycin produced from *Streptomyces atroverins*, strain Askar-SH50 show binds to the amino acid residues inside the binding pocket. Where, Met98 forms two hydrogen bonds with the NH and OH of the pyrane ring with bond length of 2.77 and 2.68 Å, Gly14 forms two hydrogen bonds with the two NH groups with bond length of 1.92 and 2.52 Å, Ser 94 form a hydrogen bond with NH of 2.24 Å, Gly96 form a hydrogen bond with OH group of 2.52 Å and Ala22 form a hydrogen bond with NH of 3.15 Å.

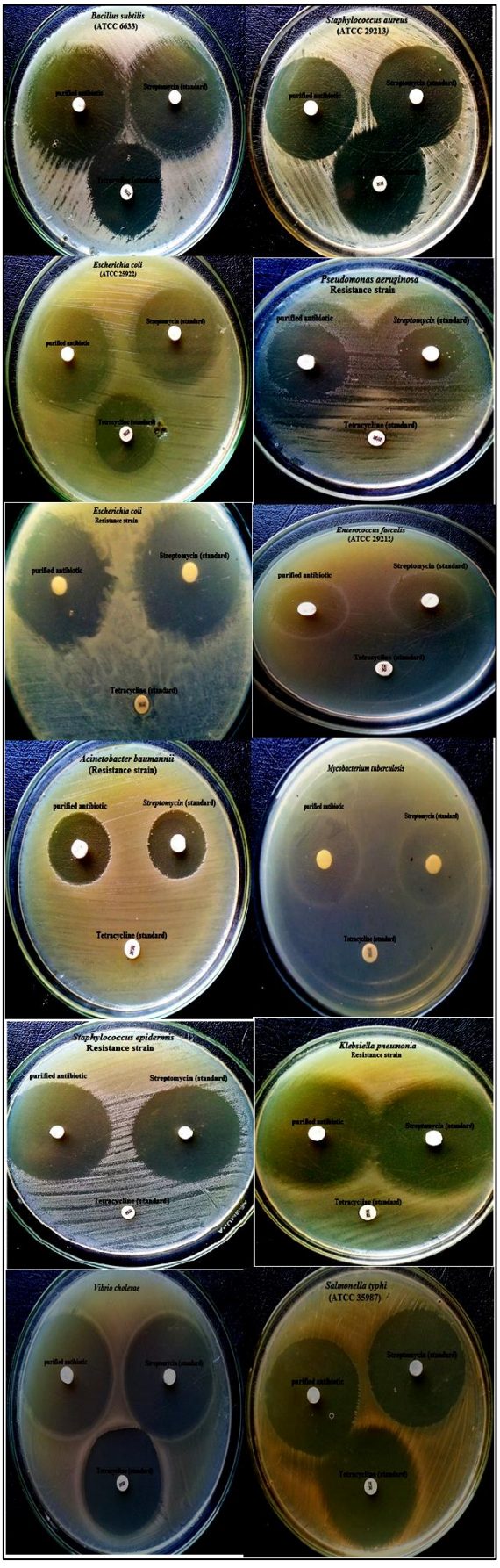


Figure 14: In vitro antimicrobial activity for Streptomycin which produced from Streptomyces atroverins, strain Askar- SH50

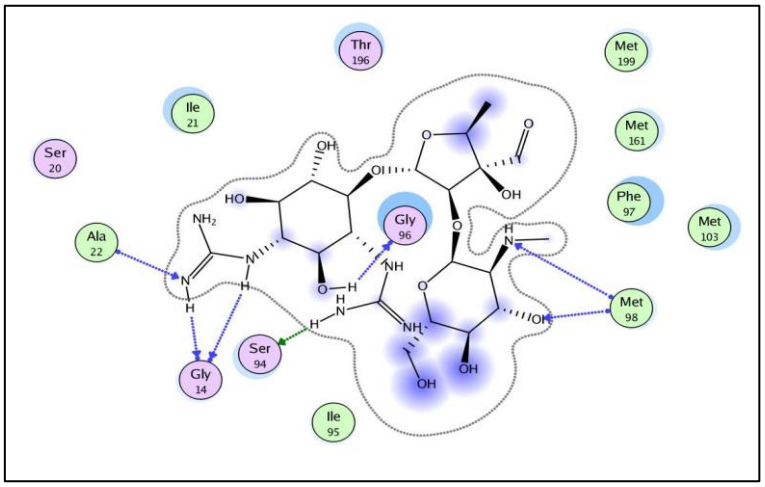


Figure 15: 2D interaction map of streptomycin produced from Streptomyces atroverins, strain Askar-SH50 inside the active site of 5JFO

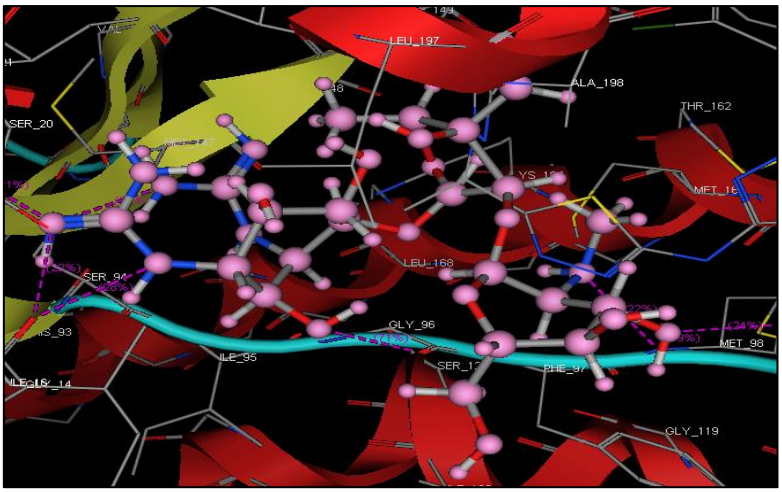


Figure 16: 3D interaction map of streptomycin produced from Streptomyces atroverins, strain Askar-SH50 inside the active site of 5JFO

CONCLUSION

In summary, the screening for bioactive metabolites that have inhibitory influences against prokaryotic and eukaryotic microorganisms from actinomycetes strains was performed. *Streptomyces atroverins* strain Askar-SH50 was isolated from marine samples to produce bioactive metabolites (streptomycin). It had been showing antimicrobial actions against Gram-positive, Gram-negative bacteria, fungi, and multi-drug resistant bacteria including *Staphylococcus aureus* (MRSA), *Staphylococcus epidermis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumonia* and *Mycobacterium tuberculosis*. The results of MIC were recorded as: 125 µg/ml, 15.62 µg/ml, 3.90 µg/ml, 62.5 µg/ml, 7.81 µg/ml, 3.90 µg/ml and 15.62 µg/ml, respectively.

The present study has opened up the new way for production streptomycin from *Streptomyces atroverins*, strain Askar-SH50 and using this natural biomolecule as anti-pulmonary tuberculosis causing by *Mycobacterium tuberculosis*. Also, it was used toward multi-drug resistant bacteria treatment. Results that, encouraging from molecular docking indicates, the produced streptomycin show binds to the amino acid residues inside the binding pocket.

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Compliance with ethical standards

Conflict of interest: The authors argue that they have no opposition of interest.

Ethical statement: This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

[1] I Chhiaki; K Naoko; K Masazumi; K Takeshi; H Naoko. *Actinomcetologica*, **2007**, 21, 27-31.
[2] CW Lo; NS Lai; HY Cheah; NK Wong; CC Ho. ASEAN Review of Biodiversity and Environmental Conservation. **2002**.
[3] P Ellaiah; T Ramana; K Raju; P Sujatha; A Sankar. *Asian J Microbiol Biotechnol Environ Sci*, **2004**, 6, 53-56.
[4] M Takizawa; R Colwell; R Hill. *Appl. Environ. Microbiol*, **1993**, 59, 997-1002.
[5] AI El-Batal; MH El-Sayed; BM Refaat; AA Askar. *Brit J Pharm Res*. **2014,** 4,(21).
[6] A Manteca; R Alvarez; N Salazar; P Yague; J Sanchez. *Appl. Environ. Microbiol*, **2008**, 74, 3877-3886.
[7] B Singh; D Mitchison. *Br Med J*, **1954**, 1, 130-132.
[8] C Kehrenberg; S Schwarz. *Antimicrob Agents Ch*, **2007**, 51, 2244-2246.
[9] SA Waksman. *Streptomycin*, its nature and practical application. 2nd edition, Williams &Wilkins Co., Baltimore, **1949**, 164-412.
[10] H Hinshaw; W Feldman; K Pfuetze**.** *Am Rev Tuberc*, **1946**, 54, 191-203.
[11] J Parsons; J Holmes; J Rojas; J Tsai; C Strauss. *J. Comput. Chem.*, **2005,** 26 1063-1068.
[12] M Ghorab; M Alkaid; M El-Gaby; N Safwat; M Elaasser; A Soliman. *Eur. J. Med. Chem.*, **2016,** 124, 299-310.
[13] P Tsao; C Leben. *Phytopathology*, **1960,** 50, 88-89.
[14] T Mincer; P Jensen; C Kauffman; W Fenical. *Appl Environ Microb*, **2002**, 68, 5005-5011.
[15] P Kaushik; N Kishore. *J. Orchid Soc*, **1991,** 5, 93-96.
[16] SA Waksman. The Actinomycetes, classification, identification and descriptions of genera and species, *Vol.II*, The Williams and Wilkins company. Baltimore.**1961**.
[17] RY WU. Bot. *Bot Bull Academia Sinica*, **1984**, 25, 111-123.
[18] E Shirling; D Gottlieb. *Int J Syst Evol Micr*, **1966**, 16, 313-340.
[19] A Kornerup, J Wanscher. Methuen Handbook of the color. edition Methuen. London.UK, ISBN 10, **1978**.
[20] H Tresner, M Davies, E Backus. Journal of Bacteriology. **1961**,81, 70–80.
[21] J Bozzola, L Russell. Electron Microscopy: Principles and Techniques for Biologists, 2nd edition, Boston Jones and Bartlett Publishers, **1999,** 542.
[22] B Becker; M Lechevalier; H Lechevalier. *Appl. Microbiol*, **1965**, 13, 236-243.
[23] M Lechevalier; H Lechevalier. *J Syst Bact*, **1970**, 4, 435-443.
[24] P Kampfer; R Kroppenstedt; W Dott. *J Gen Microbiol*, **1991**, 137, 1831-1891.
[25] J Sambrook, E Fritsch, T Maniatis. Molecular cloning: a laboratory manual, 2nd edition Cold Spring Harbor Laboratory, Cold Spring, **1989.**
[26] U Edwards; T Rogall; H Bocker; M Emade; E Bottger. *Nucleic Acid Res*, **1989**, 17, 7843-7853.
[27] T Hall. *Nucl. Acids Symp. Ser*., **1999**, 41, 95-98.
[28] R Page**.** *Comput Appl Biosci*. **1996**, 12, 357-358.
[29] J Berdy**.** CRC Handbook of Antibiotic Compounds, Vol II, CRC Press, Boca Raton, Florida, **1980.**
[30] J Berdy. CRC Handbook of Antibiotic Compounds I, CRC Press. Boca Raton, Florida, **1980.**
[31] J Berdy. *Adv. App. Microbiol.*, **1974**, 14, 309-406.
[32] H Umezawa. *Jap. J. Antibiotic Suppl*., **1977**, 30, 138-173.
[33] A Chernyshev, V Katashov, A Arzamastev, S Esipov, P Senov. Complete assignment of the signals.**1980,** 543.422, 615.332.
[34] J Berdy. *Process Biochem.* **1980,** 15, 28-35.
[35] K Cooper. Academic press New York and London. **1963,** 1-85.
[36] M Martínez-Hoyos, E Perez-Harran, G Gulten, L Encinas, D Álvarez-Gómez, E Alvarez, S Ferrer-Baraga, A García-Pérez, F Ortega, I Angulo-Bartered, EBioMedicine**., 2016,** 8,291-301.
[37] C Spatz. Basic Statistics. 5th ed **,** Brooks/Cole Publ. Co, California, USA**., 1993**, 135-161.

[38] RE Buchanan; NE Gibbson. Bergey's Manual of Determinative Bacteriology, 8th edition, Williams & Wilkins company/ Baltimore, **1974**, 327-330.
[39] S Mukherjee; P Nandi. *Ind Phytopathol*. **1995,** 8, 64-71.
[40] A Basilio; I Gonzalez; M Vicente; J Gorrochategui; A Cabello; A Gonzalez; O Genilloud**.** *J. Appl. Microbiol***, 2003**, 94, 814-823.
[41] N Sahin; A Ugur. *Turk J. Biol*., **2003**, 27, 79-84.
[42] S Augustine; S Bhavsar; M Baserisalehi; B Kapadnis. *Indian J. Exp. Biol*., **2004**, 42, 928-932.
[43] PK Jain; PC Jain Hind. *Antibiot. Bull*., **2004**, 45-46, 5-10
[44] SA Williams. Bergey's Manual of Systematic bacteriology, Vol. 4 , Williams and Wilkins. Baltimore. Hong kong. London. Sydney. **1989**.
[45] W Hensyl. Bergey's Manual of Systematic Bacteriology, 9 th Edition, Williams and Wilkins. Baltimore, **1994.**
[46] H Tresner; J Hayes; E Backus. *Appl Microbiol*., **1968**, 16, 1134-1136.
[47] M Ndonde; E Semu. *World J Microb Biot*, **2000**, 16, 595-599.
[48] A Gilman, T Rall, A Nies, P Taylor. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th edition, New York, NY. Pergamon Press1100. **1990.**
[49] https://en.wikipedia.org/wiki/Streptomycin
[50] R Crosse; R McWilliam; A Rhodes; A Dunn. *Ann Appl Biol*. **1960**, 48, 270-278.