Journal of Kyzylorda Scholarly Review

P-ISSN:3006-0265 | E-ISSN:3007-4681. Published by the Open University of Kyzylorda
Copyright © Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY).
https://bulletin.ouk.kz/index.php/bulletin/index



IN VITRO AND IN VIVO EVALUATION OF THE ANTICANCER ACTIVITY OF BACTERIAL SECONDARY METABOLITES

Ahmed Abdulrahman[1*], **Hajir Ali Shareef** [2]
[1,2]Department of biology, College of Science ,University of Kirkuk, Kirkuk, Iraq
*Corresponding author. E-mail: ahmedbio727@gmail.com

Article History:
Received: 30th Sept 2026; Revised: 20th Oct 2026; Accepted: 10th Nov 2026; Published: 27th Nov 2026

Abstract: Background: Human melanoma is an aggressive type of skin cancer. The A375 cell line, derived from human malignant melanoma tissue, possesses unique genetic characteristics, most notably the V600E mutation in the BRAF gene. The constitutive stimulation of the MAPK/ERK signaling pathway, which subsequently encourages cell survival and proliferation, is caused by this mutation. As a result, under ideal culture conditions, these cells provide a crucial in vitro model for researching how cutaneous cancers react to genetic inhibitors and specific chemical agents. Actinobacteria's exceptional ability to create bioactive metabolites has made them a prospective source of anticancer chemicals.

Objectives: This study aimed to evaluate the antineoplastic effects of the bacterial extract from S.coeruleorubidus SAIXB8 against melanoma both in vitro and in vivo.

Methods and Results: Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the bacterial extract revealed the presence of several bioactive chemical compounds with diverse biological activities. The most prominent constituents included tetracosane, hexadecane, heneicosane, ascorbic acid ester derivatives, long-chain fatty acids, and esters, which are associated with antibacterial, antioxidant, and antitumor properties, thereby explaining the high biological efficacy of the investigated bacterial extracts.

The cytotoxicity of the crude extract from the S.coeruleorubidus isolate was evaluated using the MTT assay, demonstrating the highest efficacy against the cancer cell line. The results indicated that the cytotoxic effect of the extract was directly dose-dependent; the viability of A375 melanoma cells decreased progressively from (100%) in the control group to (42.27%) at a concentration of 1000 µg/ml, achieving an inhibition rate of (57.73%). Statistical analysis via ANOVA revealed highly significant differences ($P < 0.0001$).

Furthermore, the IC50 value was determined to be 356 µg/ml, indicating that the crude extract contains promising bioactive compounds with potent antitumor activity, suggesting their potential future utility in developing therapies of natural origin.

In mice with mammary gland cancer, the in vivo investigation further showed that the bacterial extract has antineoplastic action. Tumor weight and volume gradually decreased as a result of treatment in a dose-dependent manner. When compared to the control group, the 400 mg/kg dose produced the greatest tumor inhibition rate at (59%). Furthermore, the treated mice showed no discernible toxic symptoms or behavioral alterations.

Keywords: Human Melanoma Cell, S.coeruleorubidus, Cell culture, 16S rRNA, MTT



Introduction

One of the main causes of death worldwide is cancer. It appears as a result of abnormal changes in a particular group of healthy cells, causing unchecked growth that results in a mass called a tumor. If this cancerous growth is not treated, it may spread to distant anatomical locations through the lymphatic or circulatory systems or infect nearby healthy tissues [1]. Vincristine, Camptothecin, Irinotecan, Bleomycin, Mitomycin, Dactinomycin, and Doxorubicin are among the approximately (60%) of currently used anticancer medications that are naturally produced, primarily from actinomycetes sources. Nevertheless, the clinical efficacy of numerous chemotherapeutic agents is currently hindered by the emergence of multi-drug resistance, alongside their associated cytotoxic impacts on healthy cells, which provoke severe adverse side effects in patients [2]. Consequently, there is an escalating research momentum toward identifying and designing novel oncology agents characterized by enhanced therapeutic efficacy and selective cytotoxicity against malignant cells.

The human skin tissue affected by malignant melanoma is the source of the well-known, ongoing A375 cell line. When xenografted into immunodeficient mice, these cells show strong tumorigenic potential and morphologically exhibit epithelial features, sometimes presenting spindle-shaped profiles [3]. As a result, A375 cells and their derivatives are used as a common in vitro model to assess the cytotoxicity of natural substances and secondary metabolites and to look for substances that may cause apoptosis [4].

Actinobacteria are prokaryotic, filamentous, Gram-positive bacteria, some of which are acid-fast. They often produce branching filaments with a diameter of 0.5 to 1.0 microns. The most abundant producer of antibiotics is the species Streptomyces, which also has an amazing ability to biosynthesize a variety of bioactive secondary metabolites with a wide range of uses. These metabolites are widely exploited as anticancer agents, immunosuppressants, agricultural antifungals, herbicides , anthelmintics, and insecticides Notably, the yield and expression of these compounds are heavily modulated by culture conditions and nutritional requirements [5].

Within this genus S. coeruleorubidus was initially isolated and documented in 1957 by the researcher Preobrazhenskaya in Russia. This species is characterized by its capacity to yield a broad spectrum of biologically active secondary metabolites. Primarily retrieved from terrestrial soils and occasionally from marine ecosystems it is recognized for synthesizing vital antibiotics such as Telomycin and Neomycin. The metabolites generated by S.coeruleorubidus are distinguished by their versatile bioactivities. For instance, the specific strain NAM 19 , isolated from Egyptian soils, is prominent for producing the enzyme Cholesterol oxidase flavoprotein extensively utilized in contemporary biotechnological applications [6].

Premised on the aforementioned data, the current study is designed to explore the biomedical prospects of Actinobacteria isolated from the soil of Kirkuk Governorate. This is achieved by isolating and characterizing these bacteria utilizing conventional and molecular diagnostic methodologies, followed by investigating their capacity to yield extracts with multi-targeted biological activities, specifically focusing on antineoplastic and cytotoxic efficacy against cancer cell lines. Ultimately, this research aims to contribute to the discovery of novel bioactive leads that may hold pivotal roles in developing potent therapeutic strategies to counter contemporary oncological challenges.

Materials and Method

Collection of Soil Samples and Isolation of Streptomyces spp.

Fifty soil samples were collected from various places within the city of Kirkuk. About 100 grams of dirt were taken from a depth profile of 10 to 15 cm for each sample. The rhizosphere zones around the root systems of particular plant species, especially namely Mentha (mint) and Eucalyptus.

For the isolation of Streptomyces spp. , soil samples were pretreated with CaCO3 at a ratio of 1:10 (w/w). Subsequently, a serial decimal dilution series was performed using physiological saline solution. For this purpose, 1 g of each processed soil sample was suspended in a test tube containing 9 mL of the saline solution to obtain the initial 10-1 dilution. Progressive tenfold serial dilutions were then executed to achieve a final concentration of 6-10 Aliquots of 0.5 mL from the final three dilutions were transferred into sterile Petri dishes. Each dish was then poured with approximately 20 mL of sterilized (ISP-2) agar medium. After solidification, the plates were inverted and incubated at 30°C for a duration of 7 to 14 days until distinctive bacterial colonies became visible [7].

After five days of incubation at 30 °C on ISP-2 agar medium (composition: yeast extract 4 g/L, malt extract 10 g/L, dextrose 4 g/L, pH 7.2), strain morphology was assessed.

The strain was grown on ISP4 agar medium (composition: starch 10 g/L, K_2HPO_4 1 g/L, SO_4 ,$7H_2O$ 1 g/L, $(NH_4)_2SO_4$ 2 g/L, $CaCO_3$ 2 g/L , NaCl 1 g/L, and saline solution: oat flour 20 g/L, pH 7.4) in order to evaluate microscopic morphology and Gram staining[8].

morphological, microscopic, biochemical and culture characterization

Colony macro-morphological features like colour, form, elevation, margin, surface, and opacity were recorded on nutrient agar plates. and Casein hydrolysis, citrate utilization, urease activity, catalase test, indole production, and hydrogen sulfide (H2S) production were among the biochemical tests that were performed using manually prepared media in accordance with standard protocols outlined in the literature .Depending on the experiment, incubation periods can range from three to fourteen days. For these analyses, no commercial kits were utilized. By comparing the results with the proper positive and negative controls for each experiment, the results were interpreted[9].

Genome Extraction and Molecular Characterization of Streptomyces Sp.

Genomic DNA was extracted from each Actinobacteria isolate included in this study using the Genomic DNA Extraction Kit, following the manufacturer's protocol (FavorPrep Biotech Corp ,Taiwan).

The extracted DNA was subsequently used as a template for the amplification of the 16S rRNA gene by Polymerase Chain Reaction for molecular identification of the bacterial isolates. Amplification was performed using the following primers: Forward primer F (5′-CGCGGCCTATCAGCTTGTTG-3′) and Reverse primer R (5′-CCGTACTCCCCAGGCGGGG-3′) Initial denaturation at 94 °C for 10 minutes, 35 cycles of denaturation at 94 °C for 1 minute, annealing at 59 °C for 30 seconds, and extension at 72 °C for 2 minutes, followed by a final extension step at 72 °C for 10 minutes and a hold at 4 °C were the thermal cycling conditions. To make sure there was no contamination, negative controls devoid of template DNA were used. An ABI 3130 Analyzer automated sequencer was used to sequence amplicons [10] . The obtained gene sequences were compared with reference sequences deposited in the (NCBI) database, and sequence similarity analyses were performed using the (BLAST) to determine the closest phylogenetic affiliations of the isolates. [11].

Submerged, fermentation and Ethyl Acetate Extract From the Actinobacterial Strain

The selected bioactive bacterial isolates were inoculated into 500 mL flasks containing 250 mL of (ISP-2) broth, adjusted to a pH of 7.2. The cultures were subsequently incubated at 28°C in a rotary shaking incubator operating at 40 rpm for a duration of 14 days

The fermented broth was initially filtered utilizing a 0.45 µm membrane filter. Cellular debris was subsequently separated from the culture filtrate via centrifugation at 10,000 rpm for 10 minutes to obtain the cell free supernatant. This liquid extract was then mixed with ethyl acetate at a 1:1 (v/v) ratio inside a separating funnel, and the mixture was vigorously agitated for 15 minutes. [12].

The solution was allowed to stand for an adequate duration to ensure complete phase separation between the organic solvent and the aqueous layer. The upper organic phase containing the crude extract was partitioned and collected, while the aqueous phase was discarded [13].

Subsequently, the organic solvent was evaporated using a rotary evaporator at 40°C under a constant rotation speed (rpm) to remove the solvent, yielding a brownish, oily crude residue Crude extracts were weighed, resuspended in 4 % DMSO, and stored at 4 ◦C for biological activity assays , as illustrated in Figure (3-1).

GC-MS Analysis of Streptomyces Sp. (SAIXB8) Extract

To identify the bioactive compounds in the extract, GC-MS analysis was performed using a Shimadzu QP2100 ULTRA GC-MS instrument (Shimadzu, Japan), which combines two analytical techniques integrated into a single platform for the analysis of complex chemical mixtures. This technique is fundamentally based on the vaporization of complex samples and the migration of their constituents through a capillary column containing a stationary phase, propelled by an inert carrier gas such as helium or nitrogen. The chemical compounds are separated based on differences in their boiling points and differential affinities toward the stationary phase, resulting in distinct retention times.

Subsequently, the eluted compounds enter the mass spectrometer, where the molecules undergo ionization, transforming into charged ions that fragment into smaller ionic species. The mass-to-charge ratio(m/z) of these ions is sequentially measured, enabling the determination of the chemical composition and structural characteristics of the target compounds [12]. Because of its great sensitivity and precision, GC-MS can detect and profile complex chemicals at trace quantities in the ppb and ppt levels.Furthermore, it is widely employed for both qualitative and quantitative analyses of volatile organic and inorganic compounds, establishing it as one of the most extensively utilized analytical tools in contemporary analytical chemistry [13].

In-vitro anticancer activity

The cell lines evaluated in this study comprised the human malignant melanoma cell line (A375). All experimental procedures were conducted at the Photon Center for Nanotechnology Research and Development, Baghdad, Iraq.

The cell lines were cultured in 75 cm^2 tissue culture flasks (T-flasks) containing complete RPMI-1640 growth medium supplemented with (10%) (v/v) fetal bovine serum (FBS) and (1%) (v/v) penicillin–streptomycin. Cultures were maintained in a humidified incubator at 37°C under an atmosphere of (5%) CO_2.[14] [15].

Cells were seeded at a density of 1×10^4 cells/well in a 96-well plate and allowed to adhere overnight. The following day, cells were exposed to varying concentrations of the bacterial

crude ethyl acetate extract (0.5, 1, 2.5, 5, 10, 25, 50, and 100) µg/ml of extract for 72 hours. Following treatment, each well received 10 µL of MTT solution (5 mg/ml; SEAVA, UK) and was carefully mixed. To fully dissolve the purple formazan crystals, 100 µL of DMSO was added to each well after the supernatant was removed following a 4-hour incubation period. An ELISA micro plate reader (Human, Germany) was used to measure absorbance at 570 nm in order to determine cell viability. By comparing the absorbance of cells treated with the extract with that of untreated cells, the percentage of cell viability was calculated. The results are shown as a percentage in relation to the control. [16].

In Vivo Experimental Animals and Tumor Induction

Female Swiss albino mice, aged 3–5 weeks and weighing 20-25 g, were procured from the Center for Cancer and Medical Genetics Research in Baghdad, Iraq. The experimental animals were acclimated and maintained under optimal laboratory conditions, with the temperature regulated at 28 °C and a controlled light-dark cycle. Standard rodent diet and water were provided ad libitum.

Mammary carcinogenesis was induced in the laboratory mice following an established international protocol via the administration of 7,12-Dimethylbenz[a]anthracene (DMBA). Animals received a dosage ranging between 50 and 100 mg/kg of body weight, administered either as a single dose or in split, periodic doses. Palpable tumors developed approximately two months post-induction [17].Mice that successfully developed tumors were isolated from those that did not.

Experimental Design and Grouping

A total of 30 tumor-bearing mice were selected and randomly allocated into the following experimental groups [14]. Group 1 (G1): Tumor-bearing mice treated with the bacterial extract at a dose of 100 mg/kg ,Group 2 (G2): Tumor-bearing mice treated with the bacterial extract at a dose of 200 mg/kg ,Group 3 (G3): Tumor-bearing mice treated with the bacterial extract at a dose of 300 mg/kg ,Group 4 (G4): Tumor-bearing mice treated with the bacterial extract at a dose of 400 mg/kg ,Group 5 (G5): Positive control group; tumor-bearing mice that received no treatment,Group 6 (G6): Negative control group; healthy, untreated mice.

Tumor Volume Measurement

Tumor dimensions were monitored using a digital Vernier caliper. The longitudinal length and transverse width of the tumors were measured, and the total tumor volume was calculated according to the following standard formula .

Tumor volume = a × (b)2/2

Statistical Analysis

Statistical evaluation of the data was performed using one-way analysis of variance (ANOVA) via the SPSS software package. Graphical representations and charts were generated using Microsoft Excel 2010. Quantitative results were expressed as mean ± standard deviation (SD), and statistical significance to determine distinct differences between the means was established at a threshold of $P < 0.05$ [18].

Results

Molecular, morphology, microscopy, biochemistry of the Actinomycete Strain and its Metabolites

A Streptomyces species was tentatively identified as the SAIXB8 strain [19]. Its 16S rRNA gene was amplified, sequenced, and examined using the BLASTn (Basic Local Alignment Search Tool) method in order to verify this identification. Its membership in the genus Streptomyces,

which is well-known for producing a large number of beneficial secondary metabolites, was verified by the study [20]. DNA Baser Assembler software was used to put the acquired sequences together into a single contig. With accession number PX470116.1, the partial sequence has been added to the GenBank database.

In agreement with typical macroscopic and microscopic characteristics of Streptomyces species .SAIXB8 developed a beige substrate mycelium and a brown aerial mycelium when cultivated on ISP-2 medium at 30 ◦C for five days (Fig.1a). Microscopic observations revealed Gram-positive filamentous cells forming linear chains of spores (Fig.1b), a morphology consistent with previously described Streptomyces strains [21].

After taxonomic identification, we used a number of biochemical and physiological tests to evaluate strain SAIXB8's metabolic potential. Only glucose and galactose were absorbed from the 10 carbon sources that were evaluated (Table 1), indicating a very limited carbohydrate utilization profile in contrast to other Streptomyces species that frequently metabolize a wider variety [22].

Biochemical tests showed positive reactions for urease, catalase, casein, citrate, and gelatin hydrolysis, indicating the presence of various hydrolytic activities relevant to potential ecological competitiveness and industrial applications. Negative reactions for hydrogen sulfide (H_2S) and indole production further refine the phenotypic profile of strain SAIXB8



Fig. 1. Morphological and microscopic characterization of the selected *Streptomyces* isolate showing colony morphology on agar

Journal of Kyzylorda Scholarly Review
P-ISSN:3006-0265 | E-ISSN:3007-4681
https://bulletin.ouk.kz/index.php/bulletin/index

Table 1. Physiological and biochemical characteristics of the selected *S.coeruleorubidus* SAIXB8 isolate

Characteristics	Selected *Streptomyces* isolate
Carbon source	
Glucose	+
Galactose	+
Mannose	−
Fructose	−
Sucrose	−
Lactose	−
Arabinose	−
Glycerol	−
Cellulose	−
Biochemical tests	
Casein hydrolysis	+
Catalase	+
Urease	+
Gelatin liquefaction	+
Citrate utilization	+
Indole production	−
H_2S production	−

(+) Positive reaction or growth ; (−) Negative reaction or no growth.

Figure 2. BLAST alignment of the 16S rRNA gene sequence of the studied isolate with the reference strain *S.coeruleorubidus* SAIXB8.

Score	Expect	Identities	Gaps	Strand
1058 bits(1172)	0.0	586/586(100%)	0/586(0%)	Plus/Plus

```
Query  1    GCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCC  60
Sbjct  236  ............................................................  295

Query  61   AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGC  120
Sbjct  296  ............................................................  355

Query  121  GACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCG  180
Sbjct  356  ............................................................  415

Query  181  AAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATA  240
Sbjct  416  ............................................................  475

Query  241  CGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCA  300
Sbjct  476  ............................................................  535

Query  301  CGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGA  360
Sbjct  536  ............................................................  595

Query  361  GTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGA  420
Sbjct  596  ............................................................  655

Query  421  ACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGG  480
Sbjct  656  ............................................................  715

Query  481  GAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGG  540
Sbjct  716  ............................................................  775
```

Journal of Kyzylorda Scholarly Review
P-ISSN:3006-0265 | E-ISSN:3007-4681
https://bulletin.ouk.kz/index.php/bulletin/index

```
Query  541  GCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCC  586
Sbjct  776  ..............................................  821
```

Bioactive Compounds in the Ethyl Acetate Extract were Qualitatively Detected Using GC-MS The extract contained 29 bioactive compounds, classified as amino acids, and a variety of organic compounds including alkanes, fatty acids, esters, phenols, and flavonoids. (Table 1). GC-MS full-scan chromatography and specific compound chromatography plots are included in the Supporting Information.Among the identified compounds are Initially , Cyclobutane , 1,2-diphenyl (11.60%) , a bis-cyclic aromatic compound with antioxidant and antibacterial properties, was found, indicating its active role in the extract's biological activity. L-(+)-Ascorbic acid (2,6-dihexadecanoate), a vitamin C derivative linked to dihexadecanoate , was found at 11.04%. It is known for its high antioxidant activity and its role in protecting cells from oxidation.

Heneicosane compounds were found at (4.58% , 3.27% , and 9.35%) , respectively, with retention times of 12.36 , 13.84 , and 14.29 minutes. Hexadecane was found at 5.03% , with retention times of 5.20 and 5.79 minutes. Long-chain alkanes are commonly used as indicators of fatty or waxy compounds of plant or microbial origin. Cis-10-Heptadecenoic acid (5.95%), an unsaturated fatty acid with anti-inflammatory properties and a contribution to overall biological activity, was identified. Other compounds, such as dodecane (4.40%) , tetradecane (4.75%) , and cis-vaccenic acid (4.37%) , were also detected at concentrations ranging from 2% to (5%).

These alkanes and fatty acids contribute to hydrophobic properties and antioxidant activity. Furthermore, 1,3-Dicyclohexylurea and 5,5-Diethylpentadecane (3.38%) and 0.67% , respectively, were also recorded. Eicosan (3.42%) and its retention time were also noted (11.5, 5.79) minutes, which contributes to the stability of the extract's chemical structure. Other compounds were also detected in low concentrations (less than 2%) , including Tetraethyl silicate, Undecane Phenol, and 2,4-bis-1,1-dimethylethyl, at varying percentages (2.06% , 1.92% , 0.70%) respectively.

Table 2. List of chemical components identified by GC-MS in the crude extract of *S.coeruleorubidus*

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	4.304	4.270	4.345	361683	1.92	322663	4.03	1.12	Tetraethyl silicate
2	4.503	4.450	4.535	388786	2.06	204231	2.55	1.90	Undecane
3	4.977	4.935	5.000	132287	0.70	56530	0.71	2.34	Phenol, 2,4-bis(1,1-dimethylethyl)-
4	5.145	5.105	5.165	129498	0.69	63226	0.79	2.05	Pentadecane
5	5.201	5.165	5.265	444880	2.36	188400	2.35	2.36	Eicosane
6	5.793	5.760	5.820	231745	1.23	127619	1.59	1.82	Eicosane
7	5.849	5.820	5.880	894711	4.75	687315	8.58	1.30	Dodecane
8	6.444	6.335	6.480	947799	5.03	453178	5.66	2.09	Hexadecane
9	7.077	7.030	7.135	829849	4.40	587843	7.34	1.41	Tetradecane
10	7.444	7.410	7.485	172023	0.91	85016	1.06	2.02	Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, cis-
11	7.835	7.800	7.910	294376	1.56	86503	1.08	3.40	Benzene, 1,1'-(2-butene-1,4-diyl)bis-
12	7.970	7.930	8.000	172394	0.92	82229	1.03	2.10	Heptadecane
13	8.341	8.260	8.420	2184805	11.60	749382	9.36	2.92	Cyclobutane, 1,2-diphenyl-
14	8.810	8.765	8.900	523450	2.78	236253	2.95	2.22	Hexadecane
15	9.604	9.570	9.670	169972	0.90	70469	0.88	2.41	5-Eicosene, (E)-
16	9.788	9.745	9.845	649358	3.45	266018	3.32	2.44	Heptadecane
17	10.888	10.860	10.915	191025	1.01	126211	1.58	1.51	Heptadecane
18	11.098	11.030	11.125	403066	2.14	158439	1.98	2.54	Tetracosane
19	11.181	11.125	11.240	294009	1.56	100787	1.26	2.92	Tricosanoic acid, 10,14,18,22-tetramethyl-
20	11.287	11.240	11.325	159322	0.85	71079	0.89	2.24	Phthalic acid, 5-methylhex-2-yl butyl ester
21	11.523	11.450	11.590	644960	3.42	239591	2.99	2.69	Eicosane
22	12.043	12.013	12.090	126781	0.67	70508	0.88	1.80	5,5-Diethylpentadecane
23	12.201	12.160	12.360	2079148	11.04	905352	11.31	2.30	L-(+)-Ascorbic acid 2,6-dihexadecanoate
24	12.360	12.310	12.425	615711	3.27	247176	3.09	2.49	Heneicosane
25	12.989	12.940	13.060	657391	3.38	225671	2.82	2.82	1,3-Dicyclohexylurea
26	13.844	13.780	13.965	1762178	9.35	417687	5.22	4.22	Heneicosane
27	14.295	14.235	14.370	862530	4.58	255176	3.19	3.38	Heneicosane
28	14.457	14.370	14.485	823089	4.37	276520	3.45	2.98	cis-Vaccenic acid
29	14.516	14.485	14.580	1120887	5.95	397306	4.96	2.82	cis-10-Heptadecenoic acid
30	14.943	14.900	14.975	591194	3.14	246627	3.08	2.40	Octadecanoic acid
				18838917	100.00	8005005	100.00		

Journal of Kyzylorda Scholarly Review
P-ISSN:3006-0265 | E-ISSN:3007-4681
https://bulletin.ouk.kz/index.php/bulletin/index

The Extract Suppressed the Growth of Human Skin cancer cells (A375)

The MTT assay was used to assess the extract's cytotoxic effects on cells. As illustrated in Figure (3), the extract-induced inhibition of cell proliferation was both time-dependent (48 h) and dose-dependent (0–1000 µg/mL) in (A375) cells.

The crude extract of S.coeruleorubidus exhibited a concentration-dependent inhibitory effect against A375 melanoma cells, wherein cell viability progressively declined with increasing concentrations of the extract. Specifically, after 48 hours of incubation, cell viability was (97.93%) at a concentration of 25 µg/mL and dropped to (42.27%) at 1000 µg/mL, which corresponds to an inhibition rate of (57.73)%.

One-way analysis of variance (ANOVA) revealed highly significant differences among the various concentrations ($F = 63.87$, $P < 0.0001$). These findings concur with previous studies indicating the capacity of Streptomyces extracts to suppress the growth of A375 cells by inducing apoptosis and disrupting the cell cycle. Consequently, these results confirm that the extract possesses promising bioactive compounds with potent anti-melanoma activity [23].

Table 3. Mean cell viability percentage and standard deviation of A375 cancer cells determined by the MTT assay.

Dose (µg/ml)	A375	
	Mean	**St. Dev.**
0	100	6.243568824
10	102.094387	2.513729827
25	97.93353809	2.807416265
50	101.7592851	5.521772438
100	95.61574976	4.243154406
250	75.03211391	2.715928245
500	59.44987432	2.715928245
1000	42.27590057	0.351019267

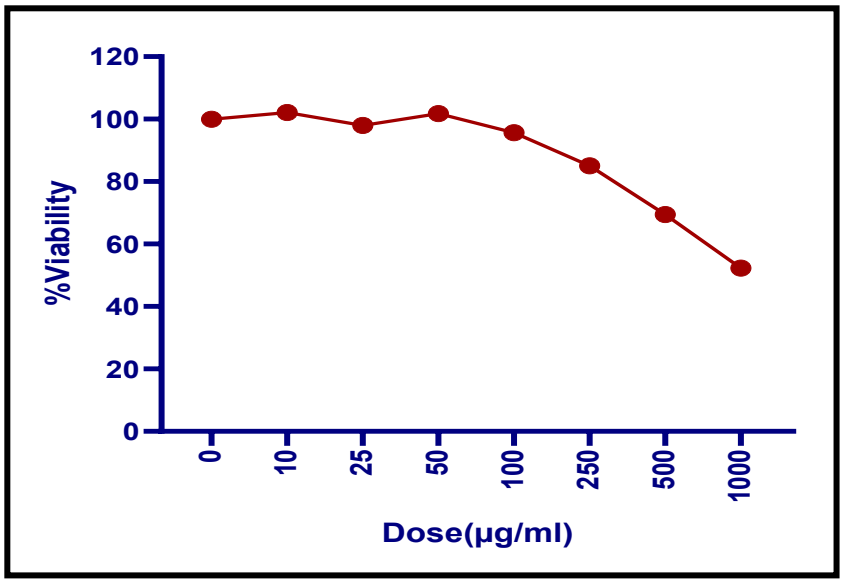

Figure 3. Effect of increasing crude extract concentrations on the viability of (A375) human melanoma cells.

Journal of Kyzylorda Scholarly Review
P-ISSN:3006-0265 | E-ISSN:3007-4681
https://bulletin.ouk.kz/index.php/bulletin/index

In Vivo Efficacy of *S.coeruleorubidus* SAIXB8 Extract Against Tumor Growth

In vivo findings revealed that the crude extract of *S.coeruleorubidus* possesses potent, dose-dependent antitumor efficacy. A statistically significant decrease ($P \leq 0.05$) in murine mammary tumor volume and weight was observed in comparison with the control cohort.

Optimally, the group administered the maximum dosage (G4) exhibited the highest therapeutic responsiveness, inducing a (59%) tumor inhibition rate, with tumor volume regressing from 19.1 ± 562 mm³ to 10.8 ± 232 mm³ . As shown in Table (3). And the following image (6) .

This therapeutic outcome is ascribed to the presence of potent bioactive constituents within the extract including phenolic compounds, terpenes, and fatty acids which exhibit antioxidant, apoptotic-inducing, and anti-angiogenic modalities.

These metrics are in robust agreement with previous literature documenting the antineoplastic potential of *Streptomyces* secondary metabolites. Notably, the absence of any overt systemic toxicity in the experimental models highlights the viability of this extract as a promising candidate for targeted anticancer drug development [23] [24].

Table 4. Doses of the Bacterial Extract and Tumor Inhibition Rates *in Vivo*

Therapeutic Response	Tumor Growth Inhibition (%)	Tumor Volume After Treatment (mm³) Mean ± SD	Tumor Volume Before Treatment (mm³) Mean ± SD	Dose (mg/kg)	Group
Slight reduction in tumor growth	14	14.1 ± 450	17.2 ± 525	100	G1
Moderate and noticeable reduction in tumor growth	28	13.3 ± 390	18.5 ± 540	200	G2
Good inhibition with marked growth retardation	41	12.4 ± 323	16.8 ± 551	300	G3
Tumor inhibition with partial regression	59	10.8 ± 232	19.1 ± 562	400	G4
Rapid and continuous tumor growth	0	21.6 ± 791	15.7 ± 510	Positive Control	G5
No tumor detected	-	-	-	Control	G6



Before treatment After treatment

Figure 4. Representative image showing the measurement of tumor mass before and after treatment.

Discussion

Actinomycetes, a unique group of bacteria known for their multitude of bioactive secondary metabolites, show significant potential in the quest for novel antibacterial and anticancer medicines [7].

Although Actinomycetes have been the source of more than half of all discovered bioactive the rate of new metabolite discovery has slowed, underlining the significance of studying previously unknown ecosystems. Actinomycetes have been studied for their unique adaptations and ability to develop medicinal chemicals with anticancer and anti-inflammatory activities.

Exploring these secondary metabolites is critical to meeting the urgent demand for new medicines, making them vital assets in the battle against diseases, particularly cancer. In this investigation, we looked at the efficacy of an ethyl acetate extract derived from a newly identified strain that had substantial cytotoxic effects during treatment.

Our investigation revealed that S.coeruleorubidus. The SAIXB8 extract effectively inhibited the proliferation of A375 cells.

The inhibitory effect was both dose- and time-dependent. The extract exhibited maximum efficacy at 1000 µg/mL after a 72-h exposure. In contrast .The Streptomyces Sp. Selective toxicity was demonstrated by the SAIXB8 extract, which killed cancer cells while sparing healthy cells. This lessens adverse drug reactions, may boost the efficacy of treatment, and enhances the quality of life for patients.

This finding suggests that the extract predominantly induces apoptosis via the extrinsic pathway. Consistently, Elmallah et al. showed that compounds such as sharkquinone, resistomycin, undecyl prodigiosin, butyl cyclopentyl prodigiosin, elloxizanone A and B, carboxyexfoliazone, and exfoliazone derived from actinomycetes isolated the were capable of inducing apoptosis in A375 cells [17].These findings from the Human malignant melanoma cell line confirmed that the extract not only inhibited growth and induced cell death in A375 cells but also exerted significant antitumor effects in vivo, inhibiting tissue processes associated with cancer growth and metastasis. Overall, the findings from both in vitro and in vivo experiments consistently demonstrate the antitumor potential of the extract. GC-MS analysis of the S.coeruleorubidus SAIXB8 extract identified 29 bioactive compounds

as major metabolites, several of which have been studied for their anticancer effects in previous research. Balhouse et al. investigated the effects of L-(+)-Ascorbic acid 2,6-dihexadecanoate on Human malignant melanoma cell line and normal Human Skin Fibroblasts These studies are essential for identifying the precise compounds causing the observed effects and determining their therapeutic indices. By resolving these issues, the SAIXB8 extract's translational potential will be strengthened and its progress toward preclinical and clinical assessments will be facilitate GC-MS analysis revealed that L-(+)-Ascorbic acid 2,6-dihexadecanoate was among the most abundant bioactive compounds detected in the extract, accounting for (11.04%) of the total identified constituents.

This compound is a stable lipophilic derivative of vitamin C and is known for its potent antioxidant properties. Previous studies have reported its ability to inhibit the proliferation of certain cancer cell lines and induce apoptotic cell death. Therefore, it may represent one of the major contributors to the anticancer activity observed in the present study. In addition, unsaturated fatty acids identified in the extract, including cis-10-Heptadecenoic acid and cis-Vaccenic acid, have been reported to influence cancer cell growth and modulate cellular signaling pathways associated with tumor development and progression, suggesting their possible synergistic contribution to the observed anticancer effects of the extract (FIGURE 6).

Conclusion

This study confirms the cytotoxic effect of the crude extract of S.coeruleorubidus isolate, exhibiting concentration-dependent selective cytotoxicity against cancer cell lines (A375), suggesting its potential as a source of anticancer compounds. The crude extract isolated from S.coeruleorubidus demonstrated in vivo antitumor activity, significantly reducing the size and weight of mouse mammary gland tumors in a dose-dependent manner, with the highest inhibition rate (59%) achieved at a dose of 400 mg/kg, without any overt toxicity observed in the treated animals. These results indicate that the extract derived from this strain represents a valuable source of pharmacological metabolites for use in anticancer applications.

References

[1] **Al-Ansari** M, Kalaiyarasi M, Almalki MA, Vijayaraghavan P (2020) Optimization of medium components for the production of antimicrobial and anticancer secondary metabolites from Streptomyces sp. AS11 isolated from the marine environment. JKSU–Science 32(3):1993–1998.

[2] **Karpiński,** T. M. & Adamczak, A. (2018) Anticancer Activity of Bacterial Proteins and Peptides. *Pharmaceutics*, 10(2):54.

[3] **Kaufman,** P. 2018. Melanoma Stage Helps Predict Survival / Everyday Health. Knott, S. R. V., Wagenblast, E., Khan, S., Kim, S. Y., Soto, M., Wagner, M.. 2018. Asparagine bioavailability governs metastasis in a model of breastcancer. Nature 554:378. doi: 10.1038/nature25465.

[4] **Hayder,** Z. H. (2022). Cytotoxic effect of asparaginase produced from Escherichia coli and conjugated with nanoparticles on skin cancer cell line (A375) (Unpublished master's thesis). University of Baghdad, College of Science, Department of Biotechnology, Baghdad, Iraq.

[5] **Lacey,** H. J., Rutledge, P. J. (2022). Recently Discovered Secondary Metabolites from Streptomyces Species. Molecules, 27 (3).

[6] **Elsayed,** E.A. and Abdelwahed, N.A.(2020). Medium optimization by response surface methodology for improved cholestrol oxidase production by a newly isolated Streptomyces rochei NAM-19 strain. BioMed Research International., 2020:1-13.

[7] **Selim,** M. S. M., Abdelhamid, S. A., & Mohamed, S. S. (2021). Secondary metabolites and biodiversity of actinomycetes. Journal of Genetic Engineering and Biotechnology, 19(72).

[8] **Coico,** R., 2006. Gram staining. Curr. Protoc. Microbiol. 00 (1). https://doi.org/10.1002/ 9780471729259.mca03cs00

[9] **Cappuccino,** J.G., Sherman, N., 2013. Microbiology: a laboratory manual. Pearson Higher Ed.

[10] **Al-zaidy,** A. K., Al-doori, A. A., & Yousif, E. H. (2023). Molecular detection of new Streptomyces spp. from Iraqi oil-contaminated soil. Iraqi Journal of Agricultural Sciences, 54(5), 1298–1304 .

[11] **Amorim,** E. A. D. F., Castro, E. J. M., da Souza, S. V., Alves, M. S., Dias, L. R. L., Melo, M. H. F., da Silva, I. M. A., Villis, P. C. M., Bonfim, M. R. Q., Falcai, A., Silva, M. R. C., Monteiro-Neto, V., Alianca, A., da Silva, L. C. N., & de Miranda, R. C. M. (2020). Antimicrobial Potential of Streptomyces ansochromogenes (PB3) Isolated from a Plant Native to the Amazon Against Pseudomonas aeruginosa. Frontiers in microbiology, 11, 574693.

[12] **Hübschmann,** H.-J. (2015). Handbook of GC–MS: Fundamentals and applications (3rd ed.). Wiley-VCH.

[13] **Tadik** onda Rama Rao, Sappidi Harshitha and Kurapati Likhitha Lahari. GAS CHROMATOGRAPHY-MASS SPECTROSCOPY: AN OVERVIEE uropean Journal of Biomedical and Pharmaceutical Scienc.Vol 10, Issue 5, 2023.

[14] **Saravana** Kumar, P.; Al-Dhabi, N.; Duraipandiyan, V.; Balachandran, C.;Praveen Kumar, P. and Gnacimuthu, S. (2014). In vitro antimicrobial, antioxidant and cytotoxic properties of Streptomyces lavendulae strain SCA5.BMC Microbio. 14:291-299.

[15] **Elgiddawy** N, Ren S, Ghattas W, Rouby W, El MA, El-Gendy A, Farghali A, Yassar A, Korri- Youssoufi H. 2021 Antimicrobial activity of cationic poly (3-hexylthiophene) nanoparticles coupled with dual fluorescent and electrochemical sensing: theragnostic prospect. Sensors 21, 1715.

[16] **Piccinini** F, Tesei A, Arienti C, Bevilacqua A. Cell counting and viability assessment of 2D and 3D cell cultures: expected reliability of the trypan blue assay. Biol Proced Online. 2017;19(1):1–12.

[17] **Fouad,** A. A., Albuali, W. H., Jresat, I., & Morsy, M. A. (2015). Protective effect of thymoquinone against DMBA-induced breast cancer in rats. *Toxicology and Industrial Health, 31*(10), 946–956.

[18] **Behnke,** J.(2006). Felix Brosius: SPSS-Programmierung Effizientes Datenmanagement und Automatisierung mit SPSS-Syntax; Andy Field Discovering statistics using SPSS. Politische Vierteljahresschrift, 47(4), 751-753.

[19] **Ayoubi,** H., Mouslim, A., Moujabbir, S., Amine, S., Azougar, I., Mouslim, J., Menggad, M., 2018. Isolation and phenotypic characterization of actinomycetes from Rabat neighborhood soil and their potential to produce bioactive compounds. Afr. J. Microbiol. Res. 12 (8), 186–191. https://doi.org/10.5897/AJMR2017.8761



[20] **Sharma,** V., Kaur, R., Salwan, R., 2021. *Streptomyces:* host for refactoring of diverse bioactive secondary metabolites. 3 Biotech 11 (7), 340. https://doi.org/10.1007/s13205-021-02872-y.

[21] Chater, K.F., 1984. Morphological and physiological differentiation in streptomyces. Microb. Dev. 89–116. https://doi.org/10.1101/0.89-115.

[22] **Krysenko,** S., Wohlleben, W., 2024. Role of carbon, nitrogen, phosphate and sulfur metabolism in secondary metabolism precursor supply in Streptomyces spp. Microorganisms 12 (8), 1571. https://doi.org/10.3390/microorganisms12081571.

[23] **Singh,** R., Singh, V., & Singh, K. P. (2023). Molecular characterization and phylogenetic analysis of actinobacteria isolated from rhizospheric soil using 16S rRNA gene sequencing. Journal of Applied Microbiology, 134(4), lxad082. https://doi.org/10.1093/jambh/lxad082

[24] **Elmallah,** M. I. Y., S. Cogo, A. A. Constantinescu, S. Elifio-Esposito, M. S. Abdelfattah, and O. Micheau. 2020. Marine Actinomycetes- Derived Secondary Metabolites Overcome Trail-Resistance via the Intrinsic Pathway Through Downregulation of Survivin and XIAP." Cells 9: 1760.

[25] **Al-Shaibani**, Muhanna Mohammed, Zin, Noraziah Mohamad, Remali, Juwairiah, Sidik, Nik Marzuki, Al-Mekhlafi, Nabil Ali, Mariappan, Vanitha, & Sukri, Asif (2025) Anticancer compounds from *Streptomyces*: insights from metagenomics and mechanistic perspective. Folia Microbiologica, 70(6), 1159–1172.