Egypt. J. Chem. **Vol. 67,** No. 4 pp. 115 - 125 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Bioactive metabolites from *Streptomyces* sp. RSE with potential anticancer and antioxidant activity

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Abstract

Marine environments are rich sources of diverse microbial life, including actinomycetes with potential for the synthesis of bioactive compounds. In this study, marine water samples were collected from Ras Sedr, Egypt, and actinomycetes were isolated using standard cultivation techniques. Small-scale fermentations were conducted, and crude extracts were obtained from the selected actinomycete isolates. Antimicrobial screening was performed to measure the inhibitory activity of the crude extracts versus a panel of bacterial strains. The most potent strain, *Streptomyces* sp. RSE, was selected based on its remarkable antimicrobial activity and identified morphologically and genetically. Furthermore, gas chromatography (GC) analysis was utilized to identify the bioactive metabolites present in the crude extract. Among the identified compounds, 3,5-Dimethyl-1,3,4-hexanetriol was determined to be a major constituent. The biological activities of this extract from *Streptomyces* sp. RSE were evaluated, focusing on antioxidant and anticancer properties. The extract exhibited significant antioxidant activity, as demonstrated by various assays. Additionally, its anticancer potential was investigated against selected cancer cell lines, revealing promising cytotoxic effects. This study highlights the potential of marine actinomycetes from Ras Sedr as valuable sources of bioactive compounds.

Keywords: Bioactive metabolites, streptomyces sp., antimicrobial, anticancer, antioxidant

1. Introduction

The ubiquitous group of bacteria known as actinomycetes is widely dispersed in natural habitats all over the planet. Actinomycetes are filamentous gram-positive bacteria with growth pattern similar to that of fungi. They are aerobic and widespread in the environment. They predominate in alkaline, dried soil [1]. DNA from actinomycetes has a high G+C concentration, with a GC% ranging from 57 to 75% [2]. Therefore, it is believed that actinomycetes are the highly effective source of the synthesis of antibiotics, secondary metabolites, and additional bioactive compounds. Every actinomycetes strain likely possesses the genetic capability to create 10 to 20 secondary metabolites, as is widely known [3], [4]. Actinomycetes create 75% of all known antibiotics, according to a significant body of research, and *Streptomyces* plays a specific role in antibiotic production. Actinomycetes are still valuable sources of novel secondary metabolites with a variety of biological functions that could eventually be used as anti-infectives, anticancer medicines, or other pharmaceutically relevant chemicals [5], [6]. Actinomycetes are widely known for producing bioactive secondary metabolites that having several health advantages, including antitumor, immunosuppressive properties, antiparasitic, antifungal, and antibacterial [7]–[9]. They reportedly also create a hormone that encourages plant development. They are utilized in bioremediation since they are well known for degrading harmful compounds. They can breakdown complex polymers

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Receive Date: 03 August 2023, Revise Date: 27 September 2023, Accept Date: 03 October 2023
DOI: https://doi.org/10.21608/ejchem.2023.226993.8357

and play a significant a function in the organic carbon recycling [10].Actinomycetes are distinguished by the development of typically branched rods or threads. The hyphae are often non-septate. The mycelium that is producing spores might be straight or spiral in shape, branching or non-branching. The spores might be round, cylindrical, or oval. *Streptomyces* (almost 70%), Nocardia, and *Micromonospora* are the most prevalent genera of Actinobacteria in soils, while *Actinoplanes* and *Streptosporangium* are also frequently discovered [11]. Actinomycetes are a large, variety of Gram-positive, aerobic, and mycelial organisms, prokaryotic organisms with a high G+C content (>55%). The preponderance of actinomycetes is saprophytic, widespread distribution of free-living bacteria in plants, water, and soil [10]. *Streptomyces* sp (GC%, 69-78) are the largest group of actinomycetes [12]. The genus *Streptomyces* was proposed by [13] and grouped according to cell wall chemical type and morphology into the family Streptomycetaceae. Numerous antibiotics that are available commercially, such as polyenes, polyethers, aminoglycosides, anthracyclins, glycopeptides, â-lactams, nucleosides, peptides, and tetracyclines, are mostly derived from streptomycetes (70%) in large quantities [14], [15].

The aim of this work was to discover bioactive compounds from sixteen actinomycete isolates from Ras Sedr with potential antimicrobial, antioxidant, and anticancer properties. One isolate, identified as Streptomyces sp. strain RSE, was selected based on antimicrobial activity and characterized. Large-scale fermentation and crude extract preparation led to the isolation of 3,5-Dimethyl-1,3,4-hexanetriol, which was evaluated for its antioxidant and antitumor activities.

2. Experimental

2.1. Marine samples collection

Samples were obtained from marine habitats at Ras Sedr (Marine water at depths of ±3-5 m and sediment-water). In an enclosed box that was dry, sterile, and hygienic, the samples were delivered aseptically to the lab and stored at 4°C until a subsequent laboratory testing.

2.2. Isolation and purification of Streptomycetes

Suspensions were plated for Streptomycetes isolation using the serial dilution method described by Hayakawa and Nonomura [16]. The day before plating, agar plates were planned and followed by incubation overnight at 37 °C to get rid of moisture layers that are on the surface of the agar (Shearer, 1987). Each dish got 0.1 ml of the appropriate dilution and was scatter with a sterile glass rod. Streptomycetes were isolated using starch nitrate agar [17]. The isolation medium for the marine water sample (50%) was seawater.

2.3. Small scale fermentation and obtaining of crude extracts

Streptomycetes isolates were fermented using media made of rice for 7 days at 30°C (150 ml of 50% natural seawater and 100 g commercial rice). Cells were incubated, ethyl acetate was used to remove the culture media from each strain, which was then filtered and decanted. In a vacuum, the organic extracts were concentrated.

2.4. Antimicrobial properties of *Streptomycetes extracts'*

To investigate the antibacterial effectiveness of different *streptomyces* extracts, experiments were conducted in Polystyrene flat plates with 96 wells. 10µl of sample extracts (final concentration of 500 g/ml) were introduced to 80 µl of lysogeny broth (LB broth), followed by 10 µl of isolated bacteria in suspension (log phase) and incubated at 37 °C for the entire night. *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* NRRLB-767 are gram-positive bacteria examples. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 are examples of gram-negative bacteria, and fungi (*Aspergillus niger* NRRLA-326) served as testing organisms. After incubation, the examined substances that had a beneficial antibacterial impact were seen as clearance in the wells, however when the tested compounds had no impact on the bacteria, the growth media in the wells appeared opaque. The pathogen in the control is left untreated. The absorbance was calculated as a mean standard deviation (SD) in a Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany) after approximately 20 hours at OD600 [18].

2.5. Identification of the most potent Streptomycetes isolate

The selected strain (RSE1), which has stronger antimicrobial properties were determined based on its physiological, morphological, and biochemical characteristics [19]–[21]. At the National Research Center in Egypt, transmission electron microscopy (HR-TEM-2100, JEOL, Japan) was conducted. Big Dye Terminator Cycle Sequencing Kit (Applied BioSystems, USA) was used for the sequencing

process. On an automated DNA sequencing device by Applied BioSystems (USA), model 3730XL, sequencing results were resolved. Information was added to the GenBank database. Using the BLAST tool, the DNA sequence was compared to the GenBank database (https://www.ncbi.nlm.nih.gov/). The bacteria's 16S rRNA gene sequences were entered into the DDBJ/EMBL/GenBank databases under accession number OQ784124.1.

2.6. Scale up Fermentation and Extraction of Bioactive substances

The selected *Streptomyces* strain (RSE1), which has a stronger antimicrobial activity, was cultured on agar plates ISP2 at for 3 days at 28°C in a scale-up fermentation experiment to produce secondary metabolites. Grams of rice were autoclaved and inoculated with a portion of the *Streptomyces* strain's well-grown agar subculture in 1 liter Erlenmeyer. Before being collected, 15 days were spent keeping the flasks at 28 °C. To obtain the crude extract, secondary metabolites were extracted from rice medium using ethyl acetate, followed by filtration and evaporation using a rotary evaporator.

2.7. purification and clarification of the bioactive compound's structure

Column chromatography was used to separate the extract using silica gel 60 F254 extract obtained by a gradient of DCM: methanol, and the drops at the column's base were gathered and fractions subject to TLC analysis. Biological tests were conducted to determine the antibacterial efficacy of the fractions obtained.

2.8. Biological activity of the bioactive compound

2.8.1. Antioxidant activity

2.8.1.1. ABTS$^{\bullet+}$ scavenging assay

The capability to neutralize free radicals was assessed by ABTS (2,2′-Azino-bis-3 ethylbenzothiazoline-6-sulfonic acid) cation radical scavenging approach [22]. The ABTS cation is dark blue-green in color and has a distinctive absorption at 734 nm. Different sample concentrations (50, 100, 200, 400, and 800 g/mL) were tested for their ability to scavenge ABTS radical cations was assessed and compared to Ascorbic Acid at equivalent concentrations.

2.8.1.2. Nitric oxide scavenging activity

According to the procedure outlined by Adithya et al. [23], the nitric oxide scavenging test was carried out. The reaction's mixture contained 0.5 mL of PBS and 2 mL of sodium nitroprusside at a concentration of 10 mM. (pH 7.4). The reaction mixture received 0.5 mL of SF2 at a concentration of (50,100,200,400, and 800 µg/mL), which was then added, shaken, and incubated for 2 hours at room temperature. A sample of 0.5 mL from the mixture was removed, mixed with 1 mL of 0.33% sulfanilic acid in a separate test tube, and left to stand for 5 minutes at room temperature. 1 mL of 0.1% naphthalene diamine chloride is then added, followed by a 30-minute incubation period at the surrounding temperature for the mixture. The absorbance was determined at 540 nm. The calculation for percent inhibition is shown above.

2.8.1.3. Lipid peroxidation inhibition assay

The method proposed by Haenen and Bast was used for determining the results of the lipid peroxidation inhibition test [24]. Thiobarbituric acid (TBA) reactive species were responsible for lipid peroxidation (LPO) activity. To produce LPO, 0.005 ml of $FeSO_4$ (0.07 M) and around 1 mL of distilled water were added to the mixture, and the combination was then incubated for 30 min. The mixture was then vortexed and heated for 60 minutes at 95°C. Next, 1.5 mL of 20% acetic acid, 1.5 mL of 0.8% (w/v) TBA in 1.1% SDS, and 0.5 mL of 20% trichloroacetic acid (TCA) were added. A different concentration of SF2 (50,100,200,400, and 800 µg/mL) was mixed similarly and incubated without TBA. Each tube was filled with 5.0 mL of butanol and centrifuged for 10 minutes at 5000 rpm after cooling. The following formula used for the % samples' average lipid peroxidation inhibition determines:

$$\% \text{ Lipid peroxidation inhibition} = [(A_0\text{-}A1)/A_0] \times 100$$

2.8.2. The effects of cytotoxicity are assessed using a variety of cell lines

For the cytotoxicity test, the cells of human liver cancer cell line (HepG cells), WI-38 cells (human lung carcinoma cells), and HEK-293 (human kidney carcinoma cell line) were planted in 96-well plates at a cell concentration of 1×10^4 cells per well in 100µl of growth medium. The viability percentage was measured as [(ODt/ODc)]x100% where ODt represents the average optical density of wells treated with the test sample and ODc represents the average optical density of cells that were not treated [25].

3. Results

3.1. Isolation of streptomycetes isolates and antimicrobial activity

Actinomycetes, particularly *Streptomyces* species, are well known for being a rich source of secondary metabolites [26], due to their wide

distribution and extraordinary capacity to produce a wide range of bioactive compounds [27]**.** In this study Sixteen streptomycetes isolates were isolated from marine habitats at Ras Sedr (Marine water at depths of ±3-5 m and sediment-water). Due to the limited treatment options for some pathogenic bacteria, primarily those that cause community- and hospital-acquired illnesses, antibiotic resistance is at a critical point [28]. Crude extracts were produced from these isolates to determine its antimicrobial activity versus *E. coli* ATCC 14169 (gram negative bacteria), *S. aureus* ATCC6538-P (gram positive bacteria), and *A. niger* NRRL A-326 (fungi). Several isolates exhibited antimicrobial activity against different bacterial isolate but one isolate (RSE1) from the 16 actinomycetes isolates showed potent antimicrobial, and were the most effective**.** While the most of crude isolate have a variable activity against bacterial isolate **(Table 1).** Despite the fact that a lot of antibiotics have been derived from a range of microbes., research is currently being done to find new antibiotics that are efficient in combating pathogenic fungus**.** Actinobacteria isolated from the marine environment have advantageous biological agents for the synthesis of compounds that fight fungus [15]. The majority of the species of *Streptomyces* are saprophytes that are frequently found in soils and play a crucial part in in the turnover of both antibiotics and complex biopolymers [29]. Chitinase enzyme was synthesized by the marine *Streptomyces* sp. DA11 isolate from South China, which was discovered to be linked to the sponge *Craniella australiensis*. It also displayed antifungal properties against Candida albicans and *Aspergillus niger* [30].

3.2. Study of the Physiological and chemo-taxanomical properties of potent Streptomyces isolate

The strain has been identified primarily by visualizing it morphology using TEM and the obtained result revealed that the strain has a rectiflexibility spore chains with a smooth spore surface, according to spore chain morphology **Figure (1)**. Tyrosine agar does not produce melanoid pigments in most cases. The spore mass is whitish in color and produces yellow diffusible pigments **(Table 2)**. Physiological and chemo-taxanomical study were presented in **Table (3)**. The obtained results revealed that, the isolate RSE1 was capable of producing melanin pigment only on tyrosine agar, while no melanin pigment was observed on peptone iron agar. The enzymatic capabilities of the isolate were also studied and the results indicated that the isolate RSE1 has proteolysis and lecithinase activity. The utilization of different carbon source was also studied and results showed that the selected isolate RES1 can utilize D glucose, D fructose and L Arabinose, while it can't consume Galactose, Sucrose, Rhamnose, D-Mannitol, D-Xylose, Raffinose, and I-Inositol. Sequencing of the most potent isolate *Streptomyces* sp. RSE1 which displayed high antimicrobial activity was carried out and the 16S rRNA gene was obtained. The (RSE) presented new *Streptomyces* sequence which was depositing in Gene Bank as *Streptomyces* sp. RSE (Accession number: OQ784124.1). The phylogenetic tree was constructed using MEGAX and presented in **Figure (2).**

Table 1: Crude extracts' antimicrobial properties form *streptomyces* isolates

Extracts from isolate no.	Antimicrobial activity (%)		
	E. coli ATCC 14169	*S. aureus* ATCC6538-P	*A. niger* NRRL A-326
RSE1	**87.89**	**92.64**	**96.05**
RSE2	99.02	NA	34.71
RSE3	NA	32.5	NA
MS1	81.90	NA	39.74
MS2	74.94	NA	34.86
MS3	8.90	NA	21.84
MS4	15.65	39.40	49.70
MS5	23.31	NA	NA
RSG1	57.55	37.40	45.61
RSG2	56.85	35.29	69.18
RSG2	63.11	NA	NA
RSP6	86.08	87.74	93.83
RSP7	61.58	NA	93.63
RL5	58.24	94.01	62.17
RL6	83.99	84.21	70.31
RS55	NA	89.21	23.07
Ciprofloxacin	98.07	96.01	-
Nystatin	-	-	98.23

NA: Not active

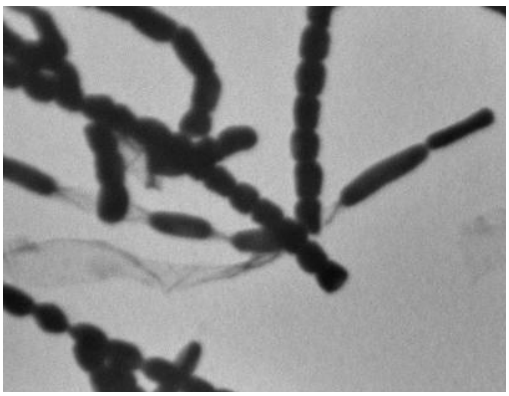


Figure (1). TEM photomicrograph showing smooth spore surface (isolate RSE1 × 25000).

Table 2. Cultural features of isolate no. RSE1 grown on various culture mediums

Medium	Color of		
	Aerial mycelium	Diffusible pigments	Substrate mycelium
1- Starch nitrate medium	Reddish gray	-ve	Grayish yellow
2- Starch-ammonium sulphate medium	Gray	-ve	Yellowish gray
3- Glycerol-asparagine medium	Gray	-ve	Yellowish gray
4- Oat-meal medium	Pale gray	-ve	Yellowish gray
5- yeast/malt extract agar medium	Whitish Gray	-ve	Pale gray
6- Czapeks medium	Gray	-ve	Pale gray

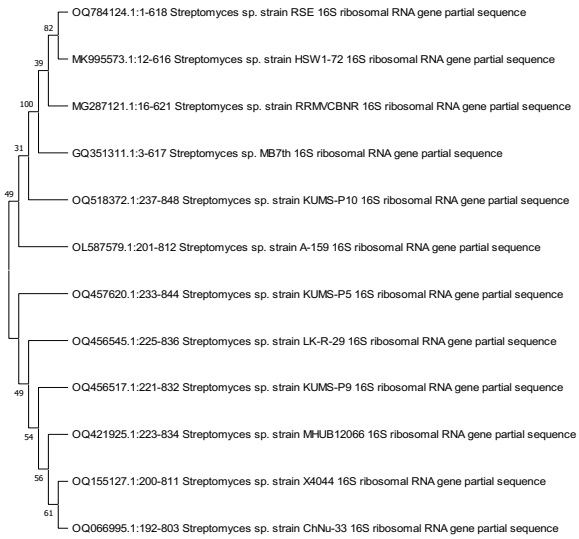


Figure (2). Phylogenetic tree of *streptomyces* sp. RSE

Table 3. Properties, both chemical and physical of the isolate RSE1

	Isolate no.	RSE1
Melanin pigment production	Pepton iron agar	-
	Tyrosine agar	+
Enzyme's activities	Proteolysis	-
	Lipolysis	-
	Lecithinase	-
Various carbon source utilization	No suger (-)	+
	D-Glucose (+)	+
	D-Fructose	+
	Sucrose	-
	Rhamnose	-
	D-Mannitol	-
	D-Xylose	-
	Raffinose	-
	I-inositol	-
	Galactose	-
	L-Arabinose	+
Nitrate reduction		+
H2S production		-
Starch hydrolysis		+
Cellulose decomposition		-
Gelatin liquification		+

3.3. Large-scale bioactive compound synthesis and purification from Streptomyces sp. RSE

The strain *Streptomyces* sp. RSE grew on medium containing rice and extracted using ethyl acetate. After evaporation, the ethyl acetate was evaporated and the obtained extract was (4.0g). the obtained extract was primarily separated into 100 fractions using Flash column chromatography. All fractions were chemically screened using TLC. Based on the TLC screening the 100 fractions were collected into 9 fractions. The obtained 9 fractions were biologically screened by evaluation of its antimicrobial activity **(Table 4).** Based upon the antimicrobial results, the most effective fraction **(F3)** was purified on Sephadex LH-20 column with a DCM:Methanol gradient mobile phase. The TLC and antimicrobial evaluation of the obtained sephadex fractions showed that only 4 fractions exhibited antimicrobial activity **(Table 5).** Among them the subfraction **(SF2)** was the most potent as it showed broad spectrum activity toward all tested microbes. The most potent sephadex subfraction **(SF2)** was further purified to remove any impurities and the

obtained compound was structurally identified using GC-MS

Table (4). Antimicrobial activity of *Streptomyces* sp. RSE flash column fractions

Extracts	Antibacterial activity (%)				Antifungal activity (%)
	Gram –ve		Gram +ve		
	E. coli ATCC25955	*P. aeruginosa* ATCC27853	*B. subtilis* ATCC6633	*S. aureus* ATCC6538-P	*A. niger* NRRL A-326
F1	34.42 ± 0.52	38.66 ± 0.98	51.82 ± 0.78	49.90 ± 0.72	NA
F2	NA	NA	NA	NA	NA
F3	72.89 ± 0.59	83.90 ± 0.85	84.90 ± 0.59	89.54 ± 0.94	63.81 ± 0.73
F4	23.89 ± 0.72	31.09 ± 0.64	48.88 ± 0.70	53.09 ± 0.92	46.90 ± 0.50
F5	NA	NA	NA	NA	13
F6	NA	NA	NA	NA	NA
F7	NA	NA	NA	NA	NA
F8	NA	NA	NA	NA	NA
F9	NA	NA	NA	NA	NA

NA : Not active

Table (5). Antimicrobial activity of *Streptomyces* sp. RSE sephadex fractions

Extracts	Antibacterial activity (%)				Antifungal activity (%)
	Gram –ve		Gram +ve		
	E. coli ATCC25955	*P.aeruginosa* ATCC27853	*B. subtilis* ATCC6633	*S. aureus* ATCC6538-P	*A. niger* NRRL A-326
SF1	42.87 ± 0.76	46.88 ± 0.85	55.84 ± 0.73	61.52 ± 0.74	43.65 ± 0.96
SF2	86.83 ± 0.97	89.65 ± 0.68	90.05 ± 0.92	93.44 ± 0.89	76.08 ± 0.85
SF3	NA	NA	NA	NA	NA
SF4	NA	NA	NA	NA	NA

NA : Not active

3.4. GC-Mass analysis of SF2

Analysis of the GC-MS of the *Streptomyces* semi pure fraction showed the existence of Benzeneacetic acid **(C1)** and Dehydromevalonic lactone **(C2)** in the active fraction as two main bioactive compounds. Based on the peak area integration, it was determined that Benzeneacetic acid has been detected at retention time 16.784 with area under the peak 33686 while Dehydromevalonic lactone has been detected at retention time 12.823 and the area under peak was 760355 **(Supplementary 1).**

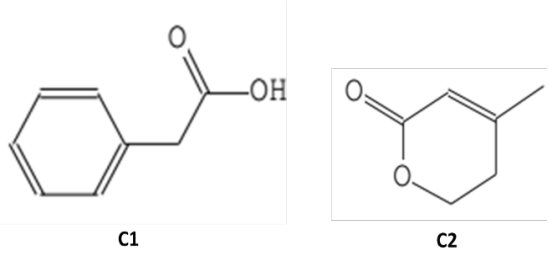
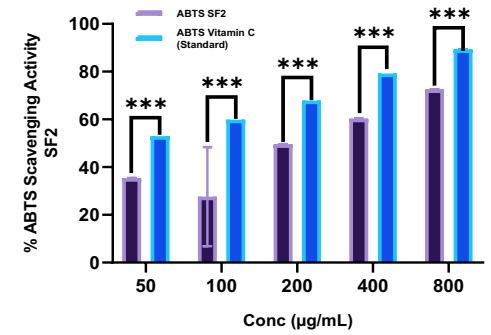


Figure (3). Chemical structure of Benzenacetic acid and Dehydromevalonic lactone

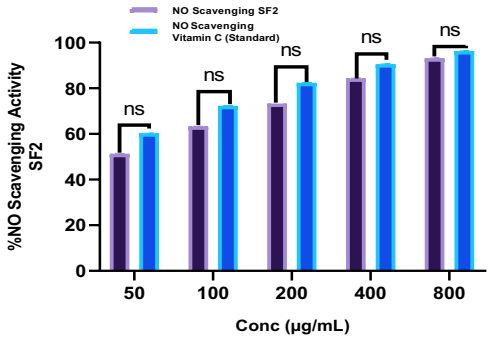
3.5. Antioxidant activity of SF2

Reactive oxygen species and reactive nitrogen species; (ROS & RNS) overproduction is an outbreak that leads to oxidative stress. An imbalance in the rates of free radical production and human body enzymatic antioxidants is referred to as oxidative stress, and it has been linked to a multitude of various health issues, such as cancer, inflammation, cardiovascular disease, and Alzheimer's disease [18]**.** Numerous studies have discovered a substantial correlation between the presence of specific chemical categories, including flavonoid and quinone derivatives and Quinones, and the antioxidant properties of endophytic and related Actinomycetes extracts [31]**.** So, in this study the ABTS discoloration method was used to test the ability of SF2 and reference drug to scavenge ABTS radicals at different concentrations **Figure (4 A)**. SF2 showed activity at the lowest concentration 50µg/ml, (35.35±0.14%) increased gradually to 72.57±0.01% by increasing concentration to 800µg/ ml; with respect to vitamin C (52.96±0.25% and 89.28±0.09% for the same concentrations, respectively). While, NO radical scavenging ability of SF2 was estimated by a SNP generating NO system. Data presented in **Figure (4 B)**, SF2 has moderate NO scavenging action compared to reference. The NO scavenging capacity was concentration dependent. Therefore, the NO scavenging action of SF2 was significantly rose from 51.26±0.03 % at 50 µg/ ml to 93.15±0.04 % at the highest concentration (800 µg/ ml) which were lower than ascorbic acid (60.26±0.04% and 96.29±0.05% at the same concentrations, respectively). Therefore, the capacity of SF2 to reduce lipid peroxidation was assessed using the thiocyanate technique. **Figure (4 C)**

showed SF2 revealed a Linoleic acid's ability to prevent peroxidation preventive**.** SF2 inhibited linoleic acid peroxidation depending on the concentration, the lowest linoleic acid peroxidation inhibition of activity (49.27±0.04%) was reported with the lowest concentration (50µg/ml). the greatest percentage (87.59±0.06%) was provided with the maximum concentration (800µg/ml). In comparison to ascorbic acid (47.25±0.03% and 91.25±0.02 for the two concentrations., respectively).



(A)



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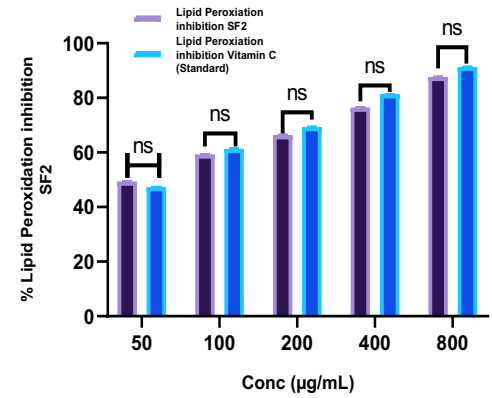
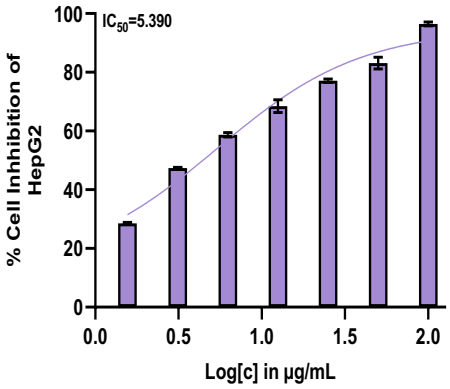


(C)

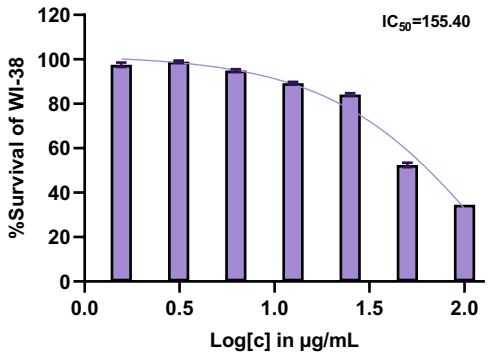
Figure (4). Scavenging activity of SF2 by (A) ABTS, (B): NO, (C): Lipid Peroxidation

3.6. Anti-tumor activity against different cell lines

According to [32]**,** marine actinobacteria are the source of many of the anticancer chemicals found in marine pharmaceuticals. These metabolites are crucial for identifying medicinal molecules. There are just a few research that has looked specifically for bioactive substances obtained from marine actinobacteria that could be employed as anticancer treatments. So, and as shown in **Figure (5)**, SF2 influenced the spread of HepG2, WI-38, and HEK-293 cells. The determined IC_{50} for cell line HepG2 was 5.390 µg/ml, whereas the IC_{50} for cell lines WI-38 and HEK-293 was 155.40 µg/ml and 78.91µg/ml, accordingly. According to Prudhomme et al. [33], pure chemical compounds taken from the marine actinobacterium *Salinispora tropica* have exhibited inhibitory effects on various malignant cell types. pure chemical compounds taken from the marine actinobacterium *Salinispora tropica* have exhibited inhibitory effects on various malignant cell types [34]. Salinosporamide A is an orally active proteasome inhibitor that, unlike the commercial proteasome inhibitor anticancer medication Bortezomib, promotes apoptosis in multiple myeloma cells [35]. Therefore, Caprolactones are novel antibiotics discovered from *Streptomyces* sp. with concomitantly low overall cytotoxicity, mild phytotoxicity, and potential efficacy against cancer cells [36]. Several reports have documented the production of bioactive metabolites with anticancer and other biological activities from bacteria [37-44] and fungi [45-56].



(A)



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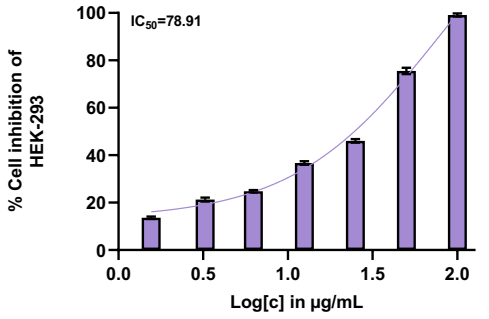

(C)

Figure (5). Evaluation of cytotoxicity of SF2 against different cell lines (A): HepG2 & (B): WI-38 & (C) HEK-293

4. Conclusion

In an effort to identify bioactive compounds that may have antimicrobial, antioxidant, and anticancer properties, sixteen actinomycetes isolates were isolated from the Ras Sedr. According to the antimicrobial testing, one isolated actinomycete has been chosen and identified depending on its morphological, physiological and biochemical properties and using 16s rDNA analysis as *Streptomyces* sp. strain RSE (Accession number: OQ784124.1). therefore, the large-scale fermentation and preparation of the crude extract, based on the antibacterial activity, and based upon its chromatographic features and GC-Mass analysis it led to the isolation of 3,5-Dimethyl-1,3,4-hexanetriol in the extract which evaluated as antioxidant and antitumor activity.

Conflicts of interest: "There are no conflicts to declare"

Acknowledgement: Supports of this work provided by national research center (NRC) and benha university, faculty of science. for the facilities provided.

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