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ANTIOXIDANT AND ANTICANCER ACTIVITY OF ACTIVE SECONDARY METABOLITES PRODUCED BY *STREPTOMYCES GRISEORUBENS*

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Natural products offer a great potential to advance the discovery of novel therapeutic agents for a variety of human diseases, particularly in cases like cancer where chemical, surgical, or hormonal treatments often come with substantial side effects. The study aimed to evaluate the bioactive potential of various Streptomyces isolates for antioxidant and anticancer activities. A total of 20 isolates of Streptomyces species were recovered from eight rhizosphere soil samples obtained in this study. Among, the isolate 506 exhibited superior antioxidant properties and substantial anticancer activity. Ethyl acetate was used to extract bioactive compounds and then were tested for free radical (DPPH) scavenging and antioxidative properties. The antioxidant activity, specifically targeting the scavenging of DPPH free radicals, for the selected Streptomyces extracts codes 1001, 1002, 1003, 1004, 506 and ascorbic acid as positive control were 15.5% ± 0.2, 21% ± 0.28, 22% ± 0.29, 21.5% ± 0.26, 28.9% ± 0.35, 47.4 ± 0.36 respectively. The sample which has shown maximum antioxidant potential was further evaluated for its anticancer activity against HepG-2 cells via MTT assay. The selected active Streptomyces strain was identified as S. griseorubens based on morphological characterization and 16S-rRNA gene sequencing, the sequence was submitted to the GenBank database under accession number OR144194. S. griseorubens 506 showed an IC50 value of 307.5 µg/mL in the anticancer assays, indicating moderate inhibitory effects on cancer cell growth. These findings highlight S. griseorubens 506 as a promising candidate for further development in therapeutic applications

Keywords: *Anticancer activity; Antioxidants; HepG2; Natural products; Streptomyces griseorubens*

INTRODUCTION

Natural products are the cornerstone of traditional medical systems and represent an important source for the development of contemporary pharmaceuticals as many modern pharmaceuticals are derived from natural sources. Moreover, the use of natural products is not limited to drugs, and their versatile applications range from biomaterial development, cosmetics, food, nutrition technology, to several industrial operations[1]. Recent research has continued to highlight the role of natural compounds in this context. Cancer, a disease of abnormal cell growth and excessive duplication, may still be the most substantial healthcare problem[2]. Chemotherapy and radiation therapy have been the largest tools in the cancer arsenal, by and large, since the dawn of cancer treatment. However, these treatments frequently produce unwanted side effects, such as damage to healthy cells, and can actually be associated with an increased probability of cancer recurrence[3]. Taken together, in consideration of these limitations and disadvantages in current cancer treatment, it is urgent to alter the chemotherapy to a more biologically-based immunotherapy with minimal or no side effects[3].

Many of the most commonly employed anticancer therapies are derived from natural sources, and cancer therapy research has greatly

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benefited from natural ingredients[4]. Such a dependence on the offerings from nature continues to underscore the importance of natural products in the ongoing evolution towards excellence in medical science[5]. Although significant advancements have been made in cancer therapy, it still remains a challenge for healthcare professionals and the pharmaceutical industry. One reason for the significance of bacteria from special biomes like the soils of coastal brine is because a large proportion of the most effective cancer chemotherapeutic drugs currently in use were either outright fabrications of compounds with structural activity relationships to powerful bioconstituents or were directly derived as natural products. These organic and inorganic compounds showcase their role in the anti-cancer armory against cancer. Therefore, natural products are increasingly being looked at for combined therapy with anticancer drugs to enhance anti-tumor efficacy and lessen systemic toxicity. The concept of metronomic chemotherapy is to provide dosages of chemotherapy in a way that will prevent tumor growth but not provide toxic levels and therefore avoid the toxicity of high-dose chemotherapy drugs to the patient[6]. Such multidisciplinary research could change how we treat cancer and offer hope for improved survival with less morbidity for persons with cancer.

Actinobacteria have been well recognized as the most industrious producers of bioactive natural products since the 1950s and are crucial for the production of secondary metabolites, including a wide array of valuable substances like antibiotics, immunosuppressive agents, enzymes, and anti-tumor compounds[7]. Thus, recent research has underscored the rising interest in Actinobacteria in discovering new natural products. Actinobacteria, especially the members of the suborder Actinomycetidae are known for their great capacity of producing a vast array of secondary metabolites. Indeed, they amount to almost half of all microbial secondary metabolites known, which makes them a very interesting reservoir for bioactive compound screenings[7]. This vast repository of secondary metabolites is mainly contributed by the Actinobacteria, wherein *Streptomyces*, a Gram-positive soil bacterium, holds the major share. *Streptomyces* undergoes a complex developmental life cycle, which includes several morphologically differentiated cell types: they grow as thin, branching hyphae to form a network of mycelia and produce spherical spores that generate long chains of spores later on[8]. The relatively simple nature of these organisms belies a complex chemistry that has been exploited to affect the social and behavioral lives of all eukaryotes, with several of their bioactive molecules now more than ever important as therapeutic agents. A number of the compounds are presently in the process of preclinical and clinical trials for a spectrum of applications[9]. These characteristics, the complicated life cycle and the collaborative nature of the *Streptomyces* genus have made the *Streptomyces* genus a promising reservoir to search new compounds.

The most promising aspect of *Streptomyces* derived compounds is that they can block the growth of tumor. Several polyketide compounds isolated from various *Streptomyces* strains have been reported to possess strong antitumor activity in the past few decades[8]. This work points to exciting new opportunities for the discovery and development of new anticancer drugs and underscores the value of investigating *Streptomyces* as a resource for anticancer agents. It is worth noting that *Streptomyces* is not confined to producing a single type of secondary metabolite, they can produce multiple types of secondary metabolite. Rather, they are a source of secondary metabolites of broad structures and biological activities, suggesting the adaptability and versatility of these bacteria with respect to biosynthesis of bioactive compounds[10]. This diversity also strengthens the reason for ongoing study of *Streptomyces* and other Actinobacteria for potential new drugs and bioproducts in the future.

S. griseorubens has mainly been found in soils[11]. Several compounds produced by *S. griseorubens* have antifungal, anti-inflammatory, and enzyme-inhibiting properties[12-13-14]. Still, not much is known about this strain's antioxidant and anticancer properties. Recent studies have continued to explore the bioactive potential of *S. griseorubens* particularly its antioxidant and anticancer properties. One such study reported an IC50 value of 55.97 µg/mL for antioxidant activity using the DPPH assay, indicating a moderate free radical scavenging capacity. Additionally, the ethyl acetate extract from *S. griseorubens* demonstrated anticancer activity

against the MCF7 breast cancer cell line, with an IC50 value of 343.34 µg/mL, as assessed by the MTT assay[15].

We hypothesize that the actinomycete *S. griseorubens*, isolated during this study, exhibits significant antioxidant and anticancer activities through the production of bioactive compounds. Thus, the present study aimed to isolate and characterize *Streptomyces* strains. Following the isolation of *Streptomyces* from the soil, the identification was performed based on morphological characteristics, such as colony appearance, pigmentation and Gram staining molecular identification was conducted using 16S rRNA sequencing, capable of producing antioxidant and anticancer metabolites. Subsequently, the cytotoxic attributes of the bioactive secondary metabolites produced by these *Streptomyces* strains were investigated.

The objectives of this study were to investigate the mechanisms by which its secondary metabolites exert these bioactive effects. Moreover, to explore the potential therapeutic applications of *S. griseorubens* in the context of drug resistance and cancer treatment. By accomplishing this, we intend to shed light on the potential biomedical applications and therapeutic significance of these naturally occurring compounds.

MATERIALS AND METHODS

Collection of samples

A total of 8 rhizosphere soil samples were collected from Elganain at Suez governate, Egypt and they were stored in the refrigerator at a temperature of 4^0C until isolation of the microbes.

Isolation of *Streptomyces* sp.

Both of starch casein agar (SCA) and nutrient agar (NA) were used as culture media for isolation of *Streptomyces* isolates. SCA was composed of (g/l): starch 10, KNO_3 2, K_2HPO_4 2, $MgSO_4 \cdot 7H_2O$ 0.05, casein 0.3, $CaCO_3$ 0.02, NaCl 2 and agar-agar 15. NA was composed (g/l): beef extract 5, peptone 5, NaCl 5 and agar-agar 15. The medium pH was adjusted to 7.0. These culture media were supplemented with Rifampicin (10 mg/ml) and nystatin (50 mg/ml) to minimize the occurrence of fungi or bacteria[16]. Isolation of *Streptomyces* was conducted by pour plate method. Serial dilutions of the collected soil were performed up to 10^{-4}. Each plate was inoculated with 0.1 ml of soil suspension and the culture medium was poured on it. The plates were manually rotated to ensure effective dispersion of the soil suspension, and were incubated at 28^o C for 7 days. The pure colonies of *Streptomyces* were subcultured onto plates of the same isolation medium and then preserved on agar slants at 4ºC.

Fermentation process and extraction of secondary metabolites

A transfer of pure colonies was made from the slants to flasks that contained 100 ml broth medium (starch casein). The cultures were then incubated in a shaker incubator at 28^o C for 8 days, the fermentation medium was filtered on filter paper and centrifuged at 1000 rpm for 15 minutes to obtain the supernatant and discard the mycelium. Extraction of the supernatant was performed by adding an equal volume of ethyl acetate and washing 3 times. Then the solvent was evaporated using a rotary evaporator to obtain the crude extract[17].

Antioxidant activity Assay

The free radical scavenging activity was determined by the method of DPPH (2, 2-diphenyl-1-picrylhydrazyl) with some modifications[18]. Using concentration (1.0 mg/1ml methanol) of the above residual extracts were dissolved in methanol and taken in test tubes separately. Ascorbic acid was used as a reference standard (positive control). Freshly prepared 0.1 mM DPPH was dissolved in methanol. DPPH (2 ml) was added to each tube of extracts (2 ml) and of standard solution (2 ml). It was shaken vigorously and they were then allowed to stand for 30 min at room temperature in a dark place. The control was prepared without adding the extract. For baseline corrections methanol was used, and at 517 nm samples absorbance (OD) was calculated. The inhibition percent was calculated according to the following equation;

$$\text{Inhibition (\%)} = \frac{\text{Abs Control} - \text{Abs Sample}}{Abs\ Control} * 100$$

Phonotypical characterization of the selected isolate *Streptomyces* 506

Colony characterization

Colony macro-morphological features like colour, form, elevation, margin, surface, and opacity were recorded on nutrient agar plates.

Microscopic characterization

Spore chain and spore surface ornamentation of *Streptomyces* were examined under light (Nikon microscope) and electron microscopes[19].

Gram staining

The Hucker method was used to perform the Gram staining on 4-day-old cultures[20].

Genotypical characterization of *Streptomyces* 506

DNA isolation and PCR amplification

DNA was extracted from the bacterial pellets according to Abd-Alla et al[21]. *Streptomyces*-specific PCR primers StrepB, 5'-ACAAGCCCTGG AAACGGGT-3' (forward), and StrepF, 5'-ACGTGT GCAGCCCAAGACA-3' (reverse) were used. 16S rRNA encoding gene was amplified by the polymerase chain reaction (PCR). PCR amplification was performed using Qiagen Proof-Start Tag Polymerase Kit (Qiagen, Hilden, Germany). The following substrates were combined in a total volume of 25 µl including about 50 ng of template DNA, 12.5 µl PCR Master Mix, 5 pmol (0.5 µl) each of forward and reverse primers and the total reaction volume was completed by 11.5 µl of water DNAase free water. This step is performed on the ice. The complete reaction mixture was incubated at an automated thermocycler TC-3000. The reaction conditions were: an initial denaturation at 95 °C for 3 min, 35 cycles of denaturation at 94 °C for 70 s, annealing at 56 °C for 40 s, and extension at 72 °C for 130 s. A final extension was conducted at 72 °C for 370s Abd-Alla et al[16]. PCR products were analyzed by electrophoresis on 1% (w/v) agarose TBE-gels (Tris-base Boric EDTA-gel) and the gels were visualized and pictured under UV light. PCR products of about (1100 bp) were purified from gel with the QIAquick gel extraction kit (Qiagen, Hilden, Germany).

DNA nucleotides sequencing

Purified PCR products were sequenced by cycle sequencing with didesoxy mediated chain termination[22]. Sequencing was done by the Biovision Company, which sent it to the Gene Analysis Unit (Macrogene Inc., Seuol, Korea) Sequencer AB-13730. For sequencing the purified PCR products, the same primers StrepB (forward) and StrepF (reverse) were used. Sequences of the 16S rRNA of isolates were first analyzed using the advanced BLAST search program at the NCBI website: http://www.ncbi.nlm.nih.gov/BLAST/ in order to assess the degree of DNA similarity. Multiple sequence alignment and molecular phylogeny was evaluated using CLUSTALW program (http://clustalw.ddbj.nig.ac.jp/top-ehtml). The phylogenetic tree was displayed using the TREE VIEW program. Phylogenetic tree derived from 16S rRNA gene sequence was generated in comparison to 16S rRNA gene sequences from different standard *Streptomyces* strains obtained from GenBank.

***In vitro* anticancer activity**

The HepG2 cell line was obtained From Vacsera in Eldoky, Egypt. The cell culture was kept at 37 °C in a humid environment containing 5% carbon dioxide, and it was given 24 hours to grow confluence. in 96-well plates for 24 hours in 200 µl PBS at a density of (104-106 cells) flat bottom HepG2 cells were seeded with minor changes After mixing DMEM with various concentrations of crude ethyl acetate extract of ***S. griseorubens*** (12.5 – 25 – 50 - 100 -200 -300 – 400 - 500 µg /mL), whereas DMSO was used as the solvent, then the mixture was incubated for 48 hours. Cells were treated with (20 µL, 5 mg/mL in PBS, pH 7.2) MTT solution and incubated at 37 °C for 4 hours before being added to 200 µL of acidic isopropanol and mixed well at room temperature for 1 hour[23]. On a scanning multi-well spectrophotometer, at 570 nm absorbance was measured, and cancer cell inhibition (%) was measured to calculate the IC50 value.

Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation. Statistical significance between groups was assessed using one-way ANOVA, followed by Tukey's post-hoc test, with a significance level of $p < 0.05$. IC50 values for anticancer activities were calculated using non-linear regression analysis in GraphPad Prism.

RESULTS AND DISCUSSION

Results

Antioxidant Activity

A total of twenty *Streptomyces* strains were isolated from 8 rhizosphere soil samples collected from Elganain, Suez, Egypt. Interestingly, the five isolates 1001, 1002, 1003, 1004, and 506, were selected for their highly antioxidant activity. The antioxidant activity of the selected *Streptomyces* extracts was evaluated using the DPPH radical scavenging assay. The results, expressed as percentage scavenging, are as follows: Extracts with codes 1001, 1002, 1003, 1004, and 506 showed scavenging activities of 15.5% ± 0.2, 21% ± 0.28, 22% ± 0.29, 21.5% ± 0.26, and 28.9% ± 0.35, respectively. The extract with code 506 exhibited the highest scavenging activity of 28.9% ± 0.35, indicating its strongest antioxidant potential among the tested extracts. The DPPH radical scavenging activity, a well-known assay for measuring the antioxidative potential of various samples, including solvent extracts and pure *Streptomyces*, was used to assess their antioxidant activity[24]. The result of the initial DPPH test with a concentration of 1.0 mg/ml is shown in **Fig. 1**. In the DPPH assay, the reduction of the free radical to DPPH-H is indicated by a color change from purple to yellow, and the inhibitory activity, expressed as a percentage, serves as a quantitative measure of the DPPH activity. Absorbance of DPPH was measured at 517 nm in the presence of different single concentrations of solvent extracts from the selected Actinomycetes strains under study. Remarkably, there were significant variations between the strains in their ability to scavenge radicals. Among these, strain 506 had the greatest capacity for DPPH free radical neutralization, while strain 1001 had the least. These findings emphasise the significant antioxidant potential of *Streptomyces* metabolites, particularly the extracted metabolites of strain 506. The efficacy of *Streptomyces* metabolites as antioxidants has garnered substantial attention in the scientific community, with multiple studies consistently affirming their significant impact in this regard[25-26].

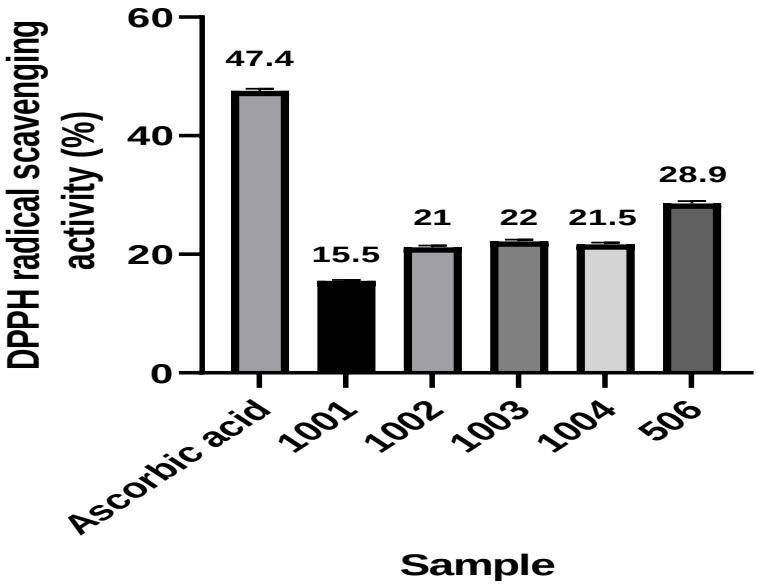

Fig. 1: This Fig. illustrates the antioxidant activity of various *Streptomyces* extracts, assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The y-axis shows the percentage of DPPH radical scavenging activity, which indicates the effectiveness of each extract in neutralizing free radicals. The x-axis lists the codes of the extracts. ,(1001, 1002, 1003, 1004, 506) and ascorbic acid as positive control were 15.5% ± 0.2, 21% ± 0.28, 22% ± 0.29, 21.5% ± 0.26, 28.9% ± 0.35, 47.4 ± 0.36 respectively Extract 506 demonstrated the highest scavenging activity at 28.9% ± 0.35, indicating its strongest antioxidant potential with $p < 0.05$.

Characterization and identification of the selected *Streptomyces*

The colony observed had an atypical morphology of *Streptomyces*, showing the typical chalky texture and mostly white color but interspersed with patches of light grey pigmentation. The colony had a webbed margin, which was especially unique. Aerial hyphae of the strain were characterized by oval spores with a spiny surface that were arranged in chains called retinacula. Aerial hyphal colours in the media used in this study ranged from dark gray to greenish-grey, light grey, to other shades of grey. In contrast, the substrate mycelia exhibited a variety of colors that were: dark brown, greenish yellow, reddish brown, pale yellow, pale olive; depending on the media used. Most interestingly, our observations revealed that this isolate did not produce any soluble or melanoid pigments as revealed in **Fig. 2**. These results provide new knowledge on the characteristics of pigment production by the lineage used herein and genetic intervention in its cellular biology, enabling a better understanding of its physiology and its metabolic pathways. Further genetic analysis was applied to confirm the identification of the selected *Streptomyces* strain. Analysis of a 1100 bp **(Fig. 3)** partial 16S rRNA gene sequence for the representative isolate showed a complete 100% homology when compared with the corresponding sequence of *Streptomyces griseorubens* in the Genbank database **(Fig. 4)**. A phylogenetic tree was then constructed to definitively place the organism in the context of documented taxa. This tree is constructed upon the alignment of numerous 16S rRNA gene sequences. The whole genetic analysis proved that the isolate chosen is *Streptomyces griseorubens*, and belongs to the family Streptomycetacea within the order Actinomycetales. The sequences of *Streptomyces griseorubens* 506 have now been deposited in GenBank nucleotide sequence database under accession number OR144194. The morphological traits and the genetic findings confirm each other, with the genetic results making a specific taxonomical classification for the isolated *Streptomyces griseorubens*. The exact identification is crucial for an insight into the phylogenetic relatedness and exploitation as a potential source in different scientific and industrial sectors.

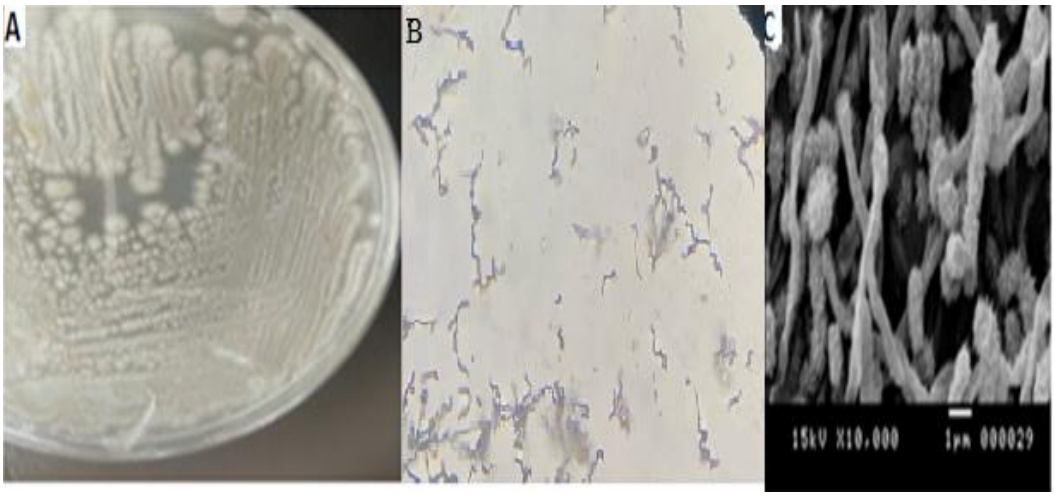

Fig. 2: Macro- and Micro-morphology of selected *Streptomyces* isolate; A) Colonies on agar plates, exhibiting a typical chalky texture and white color, interspersed with patches of light gray pigmentation. The colonies feature a webbed margin, which is a unique characteristic observed in this study, B) mycelia and spore chains under the light microscope (40x lens) and C) Spores shape under the scanning electron microscope.

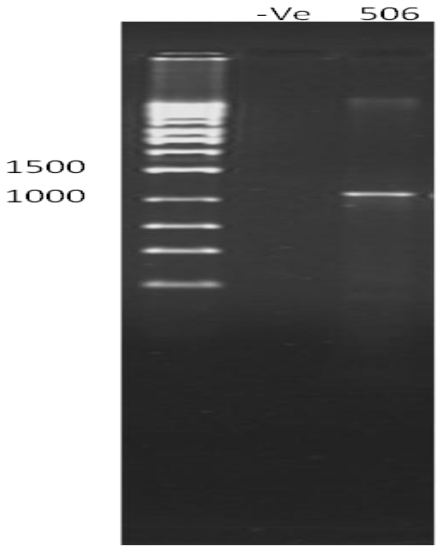

Fig. 3: Gel electrophoresis of the 16S rRNA gene of the isolate *Streptomyces* 506.

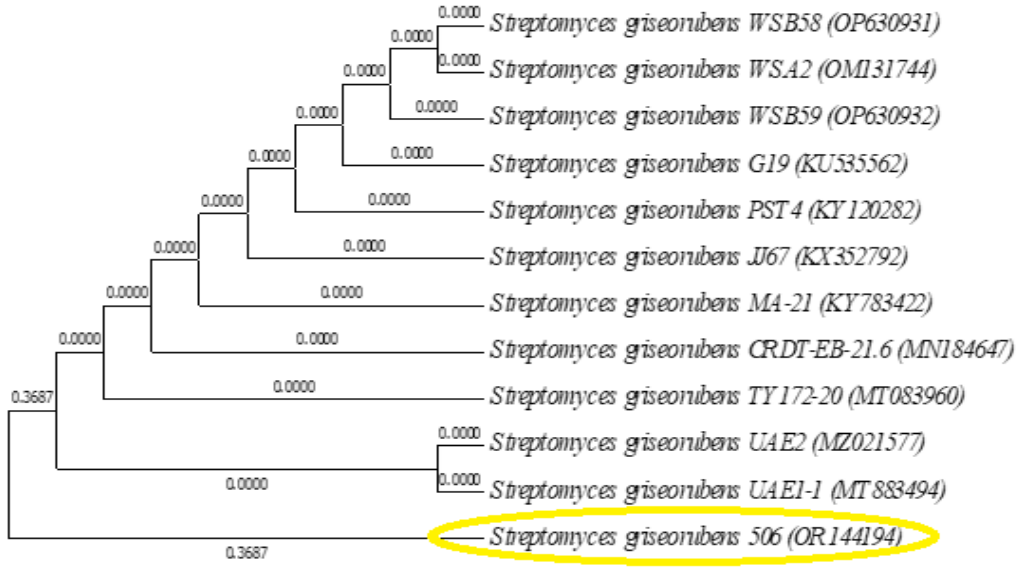

Fig. 4: The tree shows relationships among *Streptomyces* species. The representative isolate shows 100% homology with *Streptomyces griseorubens* OR144194.in the GenBank database based on 16S rRNA gene sequences showing the positions of *Streptomyces griseorubens* 506 and related strains.

Anticancer activity of the *Streptomyces* extracts

Given that strain 506 showed the best antioxidant performance among all strains in the previous screening step, this crude extract was selected to assess its anticancer activity. We assessed the anticancer capability by treating HepG2 cells with different concentrations of extract of strain 506 (12.5 ±0.34 -25 ±0.27 – 50 ±2.0 -100 ±1.0 – 200 ±1.4 -300 ±1.1 - 400±1.2 -500 ±0.3). The results showed a dose-dependent manner: the increasing of the extract concentration was followed by the decreasing of the cell viability in the HepG2 cells. The extract at the lowest concentration tested (12.5 µg/mL) affected the survival of human cells, by a mean of 13.44%, while at the higher concentration (500 µg/mL) the loss of viability was more pronounced (60.03%). Moreover, the half-maximal inhibitory concentration (IC50) of the crude ethyl acetate extract was calculated to be 307.5 µg/mL, indicating the concentration at which it effectively inhibits 50% of HepG2 cell growth **(Fig. 5&6)**. The IC50 value of 307.5 µg/mL for the crude ethyl acetate extract against HepG2 liver cancer cells demonstrated

a moderate level of anticancer activity. When compared to established chemotherapeutic agents, such as doxorubicin or paclitaxel, which typically have much lower IC50 values (in the nanomolar to low micromolar range)[27]. It is important to note that natural extracts often contain complex mixtures of compounds, which may work synergistically rather than as single agents. The IC50 value obtained here suggests that further fractionation of the extract or the identification of specific active compounds could enhance the potency the relatively high IC50 value indicates that the extract may have fewer off-target toxic effects compared to more potent drugs, potentially offering a therapeutic window for further development[15-28].

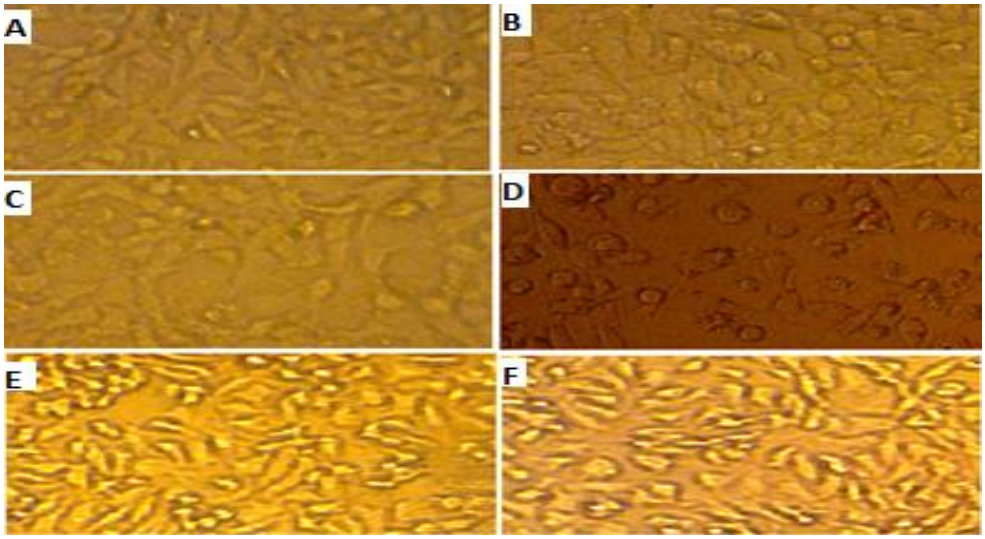

Fig. 5: Growth inhibition effect of the tested 506 on HepG2 cancer cell line. Comparison of negative control (A) and different concentrations of tested sample (12.5 -50 -200 - – 400 - 500) µg /ml (B, C, D, E, F) respectively.

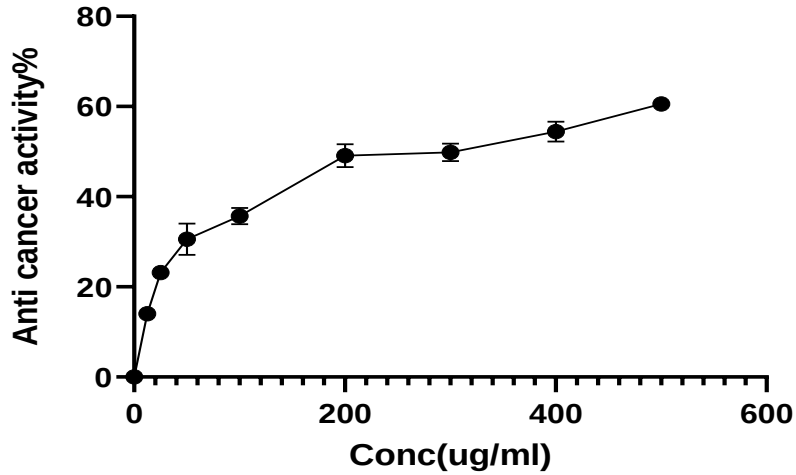

Fig. 6: **Anticancer Activity of Crude Ethyl Acetate Extract** *S. griseorubens* against HepG2 Cells. at various concentrations (12.5±0.34 -25±0.27 - 50±2.0 -100±1.0 – 200±1.4 -300±1.1 - 400±1.2 -500±0.3) µg /ml ± SE This Fig. shows the results of the MTT assay used to evaluate the anticancer activity of the crude ethyl acetate extract against HepG2 liver cancer cells. The y-axis represents the percentage of Anti-cancer activity of *S. griseorubens*, while the x-axis indicates the concentration of the extract. The IC50 value, calculated from the dose-response curve, was 307.5 µg/ml.

Regarding the cytotoxic activity of *S. griseorubens* metabolites on HepG2 cells, it is plausible that the mechanism involves apoptosis pathways. Previous research on bioactive secondary metabolites from related *Streptomyces* species, such as *S. griseorubens F8*, has shown that certain compounds induce apoptosis in cancer cells. These mechanisms may include the generation of reactive oxygen species (ROS), disruption of mitochondrial membrane potential, and activation of caspase proteins, which ultimately lead to programmed cell death. Given the complexity of these pathways, further investigation is necessary to confirm whether these mechanisms are similarly involved in HepG2 cell cytotoxicity caused by *S. griseorubens* metabolites. Such insights could be crucial for understanding their therapeutic potential and guiding future studies[29].

The study outlines several important future directions for research. To advance the development of *Streptomyces* metabolites as potential therapeutics, it is crucial to isolate and precisely characterize the active compounds responsible for the observed anti-cancer effects. This process involves employing advanced separation techniques and analytical methods. Additionally, ***in vivo*** testing is essential to assess the efficacy and safety of these compounds in animal models. Future research should focus on evaluating these metabolites in appropriate animal models to confirm their therapeutic potential and to understand their pharmacokinetics and toxicity profiles before moving to human clinical trial[30].

These data, therefore, emphasize the potential of the extract of strain 506 for its potentiality as an anticancer agent. The concentration-dependent cytotoxicity observed in HepG2 cells suggests that this extract may hold promise for further investigation and development as a candidate for cancer therapy. In the evaluation of *S. griseorubens* anticancer potential, the HepG2 cell line served as the experimental model, and a range of concentrations (ranging from 12.5 to 500 µg/mL) was employed. Our results suggest that the *S. griseorubens* extract induced cellular stress damage signal which could cause apoptosis-related cell death[31].

The capability of inducing apoptosis in cancerous cells is a cardinal characteristic sought after in any future drug candidate, anticipated for anticancer activity. Many reports have documented on role of natural products in induction of apoptosis in cancer cells, including some from Actinomycetes. For example, Al-Enazi et al. [31] supports earlier work that points to the potential of natural products to induce programmed cell death in cancer cells. Further, the concentration-dependent cytotoxic effects seen in their study are consistent with what has been seen in other chalcone substructures in the literature, with higher concentrations of cytotoxic compounds generally leading to more cell death. This phenomenon has been noted in many natural anticancer compounds. These results emphasize the need for further exploration of the cytotoxicity mechanisms caused by *S. griseorubens* extract for its consideration as a novel anticancer treatment.

Conclusion

This study indicated the powerful antioxidant as well as anticancer activities of *Streptomyces* strains isolated from rhizospheric soils. Of twenty tested strains, strain *S. griseorubens* 506 exhibited the strongest scavenging effect against DPPH radicals and thus represented to be the most promising candidate for further investigation of its antioxidative potential. The crude extract of *S. griseorubens* 506 was cytotoxic against HepG2 carcinoma cells in a dose-dependent manner and exhibited a significant inhibition in cell viability at elevated concentrations. These results not only confirmed the potential of *S. griseorubens* extracts to inhibit cancer cells but also indicated its contribution to its future pharmaceutical application. More details of the antioxidant and anticancer activity of natural products reveal new possibilities for the discovery of future therapeutic agents.

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النشاط المضاد للأكسدة والسرطان للمواد الايضية الثانوية النشطة المنتجة بواسطة استربتوميسس جريسيوريبونس *Streptomyces griseorubens*

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تُعتبر المنتجات الطبيعية مصدرًا واعدًا لتطوير عوامل علاجية جديدة لعلاج العديد من الأمراض البشرية. فهي تقدم بديلاً آمنًا مقارنة بالأساليب التقليدية لعلاج الأمراض، خاصة في حالات مثل السرطان، حيث ترتبط العلاجات الكيميائية والجراحية والهرمونية غالبًا بآثار جانبية كبيرة. لذلك تهدف هذه الدراسة إلى استكشاف الإمكانات البيولوجية لعزلات مختلفة من جنس *Streptomyces* في الأنشطة المضادة للأكسدة والمضادة للسرطان. تم عزل ٢٠ سلالة من *Streptomyces* من ثماني عينات تربة مأخوذة من منطقة الجذور. استُخدم أسيتات الإيثيل لاستخلاص المركبات النشطة بيولوجيًا من هذه العزلات، وتم اختبار المستخلصات لتحديد قدرتها على تثبيط الجزيئات الحرة (DPPH) وخصائصها المضادة للأكسدة. وقد كانت النشاطات المضادة للأكسدة التي تستهدف بشكل أساسي تثبيط DPPH، للمستخلصات المختارة من (*Streptomyces*الأكواد ١٠٠١، ١٠٠٢، ١٠٠٣، ١٠٠٤، ٥٠٦) وحمض الأسكوربيك كعنصر تحكم موجب، على التوالي: ١٥.٥% ± ٠.٢، ٢١% ± ٠.٢٨، ٢٢% ± ٠.٢٩، ٢١.٥% ± ٠.٢٦، ٢٨.٩% ± ٠.٣٥، و٤٧.٤% ± ٠.٣٦. بعد ذلك، تم تقييم العينة التي أظهرت أكبر نشاط مضاد للأكسدة لاختبار قدرتها المضادة للسرطان على خلايا HepG-2 باستخدام اختبار MTT وتم تعريف هذه العزلة النشطة على أنها *Streptomyces griseorubens* من خلال الوصف المورفولوجي والتحليل الجيني. كما أظهرت العزلة *Streptomyces griseorubens* 506، التي سجلت أعلى نشاط مضاد للأكسدة، نشاطًا مضادًا للسرطان مع قيمة IC50 بلغت ٣٠٧.٥ ميكروغرام/مل، مما يشير إلى تأثير مثبط معتدل على نمو الخلايا السرطانية. تُبرز هذه النتائج أن *Streptomyces griseorubens* 506 هى مصدر واعد لمزيد من التطوير في التطبيقات العلاجية .