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Evaluation of the Cytotoxicity of Secondary Bioactive Compounds Produced by *Streptomyces* in Soil against a Colon Cancer Cell Line

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ABSTRACT

Colorectal cancer is one of the most serious malignancies affecting humans. In this study, *Streptomyces* bioactive chemicals extracted from soil were analyzed for their anti-colorectal-cancer and antibacterial properties. A total of 100 soil samples were collected from Kerman-Iran, incubated in SCA media and the antimicrobial properties were tested using the cross-streak method. Three strains were cultured in ISP4 medium to obtain secondary bioactive compounds. After studying the effects of the bioactive compounds on the HT29 and human foreskin fibroblast (HFF) cell lines, the expression of the *p53*, *p21*, *BAX*, *BCL2*, *Casp3* and *Casp8* genes was analyzed by real-time PCR and flow cytometry to detect the presence of apoptosis.The isolates show high degree of identification with *Streptomyces rochei*, *Streptomyces fungicidicus* and *Streptomyces maritimus* due to 16SrDNA sequence homology. Compared to HT-29 cells, *Streptomyces* extracts had lower cytotoxicity against normal cells (SI=5.88), followed by HFF (SI=4.14). The cell lines demonstrated a dose-dependent significant increase in DNA fragmentation, an increase in the proportion of cells in sub-G1 phase and caused G2/M cell cycle arrest in HT-29 and HFF cells.The bacterial extracts obtained displayed strong antibacterial properties and inhibited the proliferation of HT-29 and HFF cell lines. The treated cells exhibited morphological changes caused by the activation of caspase and *p53/p21* proteins. This confirms that *Streptomyces*-induced apoptosis is mediated by the activation of *p21/p53*. Anti-apoptotic *Bcl-2* gene expression was downregulated by treatment with the extracts. Further studies are needed to understand the antimicrobial properties of *Streptomyces*.

Keywords: Colorectal cancer, *Streptomyces*, HT-29 cell line, HFF cell line, *p53/p21* proteins

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Introduction

Cancer, the second most common cause of death in human society after cardiovascular disease, is defined as the uncontrolled proliferation of abnormal human cells that can infiltrate other organs. Depending on the tissue affected, different types of cancer can manifest themselves in various ways and behave differently. As a result, not only the symptoms change, but also the rate of growth, the type of treatment and the response to it. One of the most serious cancers that can affect people and in some cases even lead to death is colorectal cancer (CRC), the second most deadly cancer. Global incidence and mortality are likely to increase in the coming decades. Although the number of deaths related to colorectal cancer is very high in high-income countries, the incidence and deaths related to colorectal cancer are also increasing in developing countries (1).

Epidemiological studies have now revealed that there is resistance to the standard drugs used to treat this malignant disease. Since typical drugs such as chemotherapy and radiation drugs have so many unfavorable and even carcinogenic effects on healthy cells, researchers are increasingly looking for drugs produced from natural sources and microorganisms, especially bacteria (2).

On the other hand, several statistics show that antibiotic resistance is increasing worldwide for various reasons. Antibiotic resistance is the emergence of new strains of antibiotic-resistant bacteria that are capable of killing people from a simple disease that was previously easily treatable. The World Health Organization recently described the increase in resistance to antibiotics as a "serious global threat" that could become the biggest problem for human society by 2050. It is important to note that CRC patients undergoing chemotherapy or surgery are more likely to develop persistent broad-spectrum infections and sepsis caused by multidrug-resistant strains (3).

Nature is an interesting source of new therapeutic agents as it represents a reservoir of secondary metabolites with a great chemical diversity. Currently, about 60% of therapeutic drugs are of natural origin. These resources include animals, plants, marine organisms and microorganisms. Among the various microbial sources, *Streptomyces* and Bacilli are particularly known to produce large amounts of unique and biologically active compounds (4). *Streptomyces* are gram-positive, filamentous bacteria that can produce various antibiotics and other important drugs for the treatment of bacterial and fungal infections, cancer and heart disease. Due to the production of more than 10,000 types of bioactive compounds among the 23,000 active compounds produced by microorganisms, members of the *Actinobacteria* phylum account for about 43% of all metabolites produced by microbes. Inorganic substances produced by bacteria, fungi or plants that are actively involved in the growth, development or reproduction of an organism are called secondary metabolites, which are often also reffered to as special metabolites, toxins, by-products, or natural products. About 75% of the bioactive chemicals of therapeutic importance are produced by the genus *Streptomyces* (5).

Studies have shown that the metabolites produced by bacteria are important sources of drugs for the treatment of resistant diseases. Bleomycin, actinomycin and mitomycin are examples of antitumor antibiotics that are naturally produced by the bacterium *Streptomyces* and are among the most effective anticancer drugs (6). The effect of these drugs is not dependent on the cell cycle and is exerted by interrupting the synthesis of DNA and RNA synthesis. Considering the emergence of antibiotic resistance

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in recent decades, which has been classified as a pervasive global risk by the WHO, and the increasing adverse side effects and resistance to cancer chemotherapy drugs, this study aimed to evaluate the antibacterial and anticancer properties of *Streptomyces* agents extracted from the soil of different regions of Kerman province to assess the possibility of killing two birds with one stone.

Materials and methods

Isolation of *Streptomyces*

Totally, 100 soil samples were taken directly with sterile spatulas from the surface layers and from a depth of 15 and 20 cm of the soil at different locations in Kerman Province to isolate the target bacteria. The samples were then packed in pre-sterilized cellophane bags. To isolate *Streptomyces* species, serial dilutions were prepared and centrifuged at 150 rpm for 30 minutes after drying the samples. Then, 0.2 ml of the dilutions were cultured in starch-casein agar (SCA) (Merck, Germany) and incubated at 30 °C for 7 days. During this period, the culture media were examined for growth or lack of growth and fungal infections. Colonies suspected to be *Streptomyces* based on morphological, cultural, physiological, and biochemical characteristics were identified, subcultured in SCA medium and incubated at 30 ° C for 2 weeks to isolate *Streptomyces* strains (7-9). In addition, reference bacteria such as *Escherichia coli* (ATCC 25522), *Staphylococcus aureus (*ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 12541), *Candida albicans* (ATCC 10231), and *Acinetobacter Baumanii* (ATCC 17978) were obtained from the Pasteur Institute (Tehran, Iran). For molecular identification of the isolates, 16S rRNA sequences were amplified with two universal primers, 1492R (5′-GGTTACCTTGTTACGACTT-3′) and Eubac27F (5′-AGAGTTTGATCCTGGCTCAG-3′), and sequenced using the Sanger method (Bioneer, South Korea), according to the study by Lee et al (10). Bio Edit software was used to organize the sequence information, and the blast tool on the National Center for Biotechnology Information (NCBI) website was used to analyze the results. Using the neighbor-joining approach to find the closest match of type strain and bootstrap values based on 1000 replicates, the phylogenetic relationship of the isolates was determined using MEGA software version 7.0.

Screening of *Streptomyces* antibacterial activity

With some minor adaptations, the streak culture technique was used to study the antibacterial properties of *Streptomyces* strains on indicator bacteria. The isolated strains were cultured on Mueller-Hinton agar plates and incubated at 28 °C for 7 days. After the bacteria grew in the center of the plates, common pathogenic bacteria were cultured with the primary bacterial samples and incubated in an incubator at 37 °C (11). Plates containing SCA medium (Himedia, India) were inoculated with fresh cultures of the recovered *Streptomyces* strains, and the plates were then incubated at 30 °C for 48 hours. The inhibitor test was performed with the above mentioned reference microorganisms. After incubating the cultures for a full day at 37 °C, the inhibition zones were analyzed and categorized as negative, + (less than 50% growth inhibition), ++ (more than 50% growth inhibition), and +++ (more than 50% growth inhibition). Strains with remarkable antibacterial activity were selected for further investigation.

Extraction of *Streptomyces* secondary metabolites

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Using GC mass and following previous studies, the secondary metabolites of the isolates with the highest antibacterial activity were isolated (12), with slight modifications. In summary, 2000 ml Erlenmeyer flasks containing 200 ml ISP1 broth (Merck, Germany) supplemented with 1% (w/v) magnesium and glucose were inoculated with *Streptomyces* spores (10^7/ml). The medium was incubated for seven days at 30 °C on an orbital shaker at 200 rpm. The mycelium and supernatant were then separated after the culture broth was filtered. Ethyl acetate solution was added to the cell-free supernatant in a 1:1 ratio and shaken rapidly for one hour. Using a rotary evaporator, the organic phase was separated and evaporated until it was completely dry. The remaining extract was measured, mixed with 5 milliliters of ethyl acetate and then cooled at 4 degrees Celsius. After filtering, the extract was subjected to a freeze-drying procedure.

Selection of the strongest metabolite-producing strains

In this method, the isolated Actinomycetes bacteria were first cultured in the center of the plate in an SCA culture medium and placed in an incubator at 30ºC for 48 hours to grow sufficiently and release the antimicrobial material in the medium. Then, a 0.5 McFarland dilution of each strain was prepared and connected perpendicular to the previous culture from bottom to top to the upper half of the plate where the desired bacteria were mass cultured in that area. The cultured plates were then stored at 30ºC for 48 hours. Finally, the diameter of the no growth zone was checked and the bacteria that produce the largest diameter of the no growth zone were selected as the strain with the highest production of secondary metabolites. Thus, 3 strains were selected as the strongest producers of secondary metabolites.

***In vitro* cytotoxic activity**

The strongest *Streptomyces* isolates were utilized to produce extracts, indicating strong antibacterial activity and possible cytotoxicity, as revealed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric MTT assay. Human colorectal adenocarcinoma (HT-29) and human foreskin fibroblast (HFF) cell lines were donated from the Iranian Biological Resource Center (IBRC) in Tehran, Iran. The cells were cultured in 15% FBS, 1% streptomycin and penicillin-containing RMPI 1640 medium before being flasked and incubated at 37 °C, 95% humidity and 5% CO2.

Cells were then planted at a density of 5×104 cells/well in a sterile 96-well plate and incubated for a full day. Cells were treated with serial dilutions of *Streptomyces* extracts (0–400 µg/mL) and incubated for a full 72 hours. The negative control was dimethyl sulfoxide (DMSO). Subsequently, the wells were filled with 20 µL of a 5 mg/mL MTT solution (Sigma, Germany) and incubated for 4 hours at 37 °C in a humid environment with 5% CO2. After aspirating the medium, 200 µL of DMSO was added to each well and pipetted with care. The formazan formed was measured spectrophotometrically at 570 nm, and the percentage of cell viability was calculated using the previously mentioned method (13). The IC50 values (50% inhibitory concentration of the purified extract) were determined on the basis of the cell line diagrams shown. VERO cells were used as a model for typical cells.

Evaluation of apoptosis induction potential

A co-staining assay with annexin V and FITC/PI (fluorescein isothiocyanate/propidium iodide) was utilized to assess the apoptotic effect of the extracts on HT-29 and HFF cells (14). For this purpose, HT-29 and HFF cells were cultured at 1×10^5 cells/mL in 24-well plates in the presence of various concentrations of isolated *Streptomyces* extracts (0–400 µg/mL) for 48 hours. At the end of incubation,

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cells were harvested and centrifuged at 15000 rpm for 10 minutes. Next, 50 µL binding buffer containing 0.5 µL Annexin V-FITC was added to the pellets and incubated for one hour in the dark at 4 °C. Then, 200 µL binding buffer containing 50 µg/mL propidium iodide was added to the cell suspension and incubated for 5 minutes. Cell apoptosis was analyzed using a Navios Ex flow cytometry system (Beckman Coulter, USA).

Effect of extract on cell cycle analysis

In the present study, the cell cycle was examined to evaluate the potential of the extracts to reduce apoptotic cell death. For 48 hours, HT-29 and HFF cell lines were grown at a density of 1×105 cells/mL in 12-well plates with different amounts of isolated *Streptomyces* extracts (125–1000 µg/mL). The cells were then purified, centrifuged at 15,000 rpm for 10 minutes, and then resuspended in 70% ethanol at 4 °C for three hours to fix them. After purification, the cells were centrifuged with phosphate-buffered saline (PBS) for 10 minutes at 15,000 rpm. After shaking the pellet, it was resuspended in 500 µl PBS containing 20 µg/ml DAPI (4′,6-diamidino-2-phenylindole) (Sigma, Germany), 20 µg/ml RNase A and Triton X-100 (0.1%). After a 15-minute dark incubation at room temperature, the suspension was filtered through a 40 µM mesh filter without being washed. Finally, the cell cycle profile was determined using a Navios Ex flow cytometer (Beckman Coulter, USA).

Study of expression of target genes

To determine the probable mechanism of cytotoxicity of the extract, the effects of *Streptomyces* secondary metabolites on the *p53, p21, BAX, BCL2, Casp3* and *Casp8* genes were investigated. Briefly, RPMI 1640 medium was used to culture HT29 or HFF cells under the above conditions. Then, different concentrations (0–400 µg/ml) of *Streptomyces* extracts were added to the culture and incubated for 48 hours. To investigate the expression of target genes, RNA isolation and cDNA synthesis were performed using an RNA purification kit (QIAGEN, Germany) and a Biotechrabbit™ synthesis cDNA Kit (Biotechrabbit, Germany), respectively. Spectrophotometry was used to measure the concentration of extracted RNA was measured (Nanodrop, Eppendorf, Germany). Subsequently, the expression of the genes was determined using specific primers with the real-time PCR method according to the instructions of the QIAGEN One-Step RT kit (QIAGEN, Germany) (Table 1).

Table 1. The sequence of used primers for quantitative real-time RT-PCR.

Gene	Primers sequences
SLC7A11	*Forward:* TGGGTGGAACTGCTCGTAA *Reverse*: AAATCTGGATCCGGGCACT
HMOX1	*Forward:* GAACCAGCCTGAACTAGCC *Reverse:* ACAAGGAAGCCATCACCAG
GAPDH	*Forward:* CAGAACATCATCCCAGCCTCC *Reverse: TTGGCAGGTTTCTCAAGACGG*

Statistical analysis

The statistical study was conducted with the program SPSS from IBM, version 18.0. Tukey's test and a one-way ANOVA were utilized to compare the groups. Statistical significance was considered acceptable with a P value<0.05.

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Results

due to the emergence of multidrug-resistant bacteria and the adverse effects of chemotherapy drugs, the need arose to discover new and potent sources of anticancer and antibacterial agents with unique modes of activity. Scientists are interested in *Streptomyces* sp. because it produces secondary metabolites with antibacterial and anticancer activity.

Isolation of potent *Streptomyces*

In this work, 100 soil samples from Kerman region of Iran were used to extract 89 *Streptomyces* strains. Based on conventional morphological and biochemical techniques, the isolates were identified. The antibacterial activities of the isolated *Streptomyces* were then investigated in comparison with reference strains. Three isolates (NO. 42, 98 and 100) indicated promising broad-spectrum activity against indicator strains. No surprisingly, 57.30% (N=51) of the isolated strains exhibited antibacterial activity against at least one of the reference bacteria. Based on 16S rDNA sequence homology analysis, the isolated bacteria were found to have 64% and 99.9% similarity to *Streptomyces rochei*, *Streptomyces fungicidicus* and *Streptomyces maritimus*, rerspectively (Figure 1). There are many reports on the antimicrobial and anticancer activity of *S. rochei* (15-17), *S. fungicidicus* (18, 19) and *S. maritimus* (20), which are in agreement with our results.

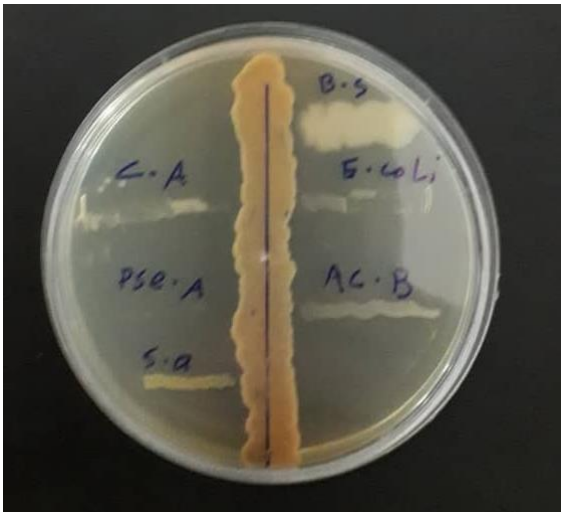

Fig. 1. The culture of isolated microorganisms includes *Escherichia coli* (*E.coli*), *Staphylococcus aureus(S.a), Pseudomonas aeruginosa(Pse. A), Bacillus subtilis(B.S), Candida albicans(C.A),* and *Acinetobacter baumanii (AC.B).*

***In vitro* antitumor activity**

The development of anticancer agents from novel resources that could be included in new therapeutic drugs has been the subject of several studies. A reliable and endless source of many bioactive chemicals with potent antibacterial, anticancer and antifungal activity is the genus *Streptomyces*. In the current study, the *in vitro* cytotoxicity of isolated *Streptomyces* extracts against HT29 and HFF cell lines was investigated using the MTT assay method. The percentage of cells after staining was classified into four quadrants including early apoptotic (Annexin V+/PI-), late apoptotic (Annexin V+/PI+), necrotic (Annexin V−/PI+) and live cells (Annexin V−/PI-). Statistical analysis of the treatments with the crude extracts of *Streptomyces*

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demonstrated different percentages of apoptotic cells (early apoptotic cells + late apoptotic cells), but overall they were able to induce apoptosis in both cell lines (Figure 2). The apoptotic effects of the ethyl acetate extracts of the isolates exhibited a dose-dependent effect on the cell lines. The ethyl acetate extract of Strain-100 represented the strongest effect against both cell lines, with the lowest IC_{50} measured at 63.7 ± 17.10 µg/mL for HT-29 and 78.11 ±6.31 µg/mL for HFF cells. Strain-98 extract showed higher IC_{50} values in HT-29 cells (115.20 ± 15.31 µg/mL) and HFF cells (211 ± 23.1 µg/mL). The IC_{50} values for Strain-42 extract were 311 ± 18.78 µg/mL for HT-29 and 278 ± 17.52 µg/mL for HFF cells. It was found that the extracts indicated a stronger apoptotic effect in both cell lines, whereas the effect was lower in normal cells. Calculation of the selectivity index (SI) revealed that the *Streptomyces* extracts exerted less cytotoxicity on normal cells than on HT-29 cells (SI=5.88), followed by HFF (SI=4.14). These findings suggest that these soil-isolated *Streptomyces* extracts could induce apoptosis in human cancer cell lines, which is consistent with previous studies (21).

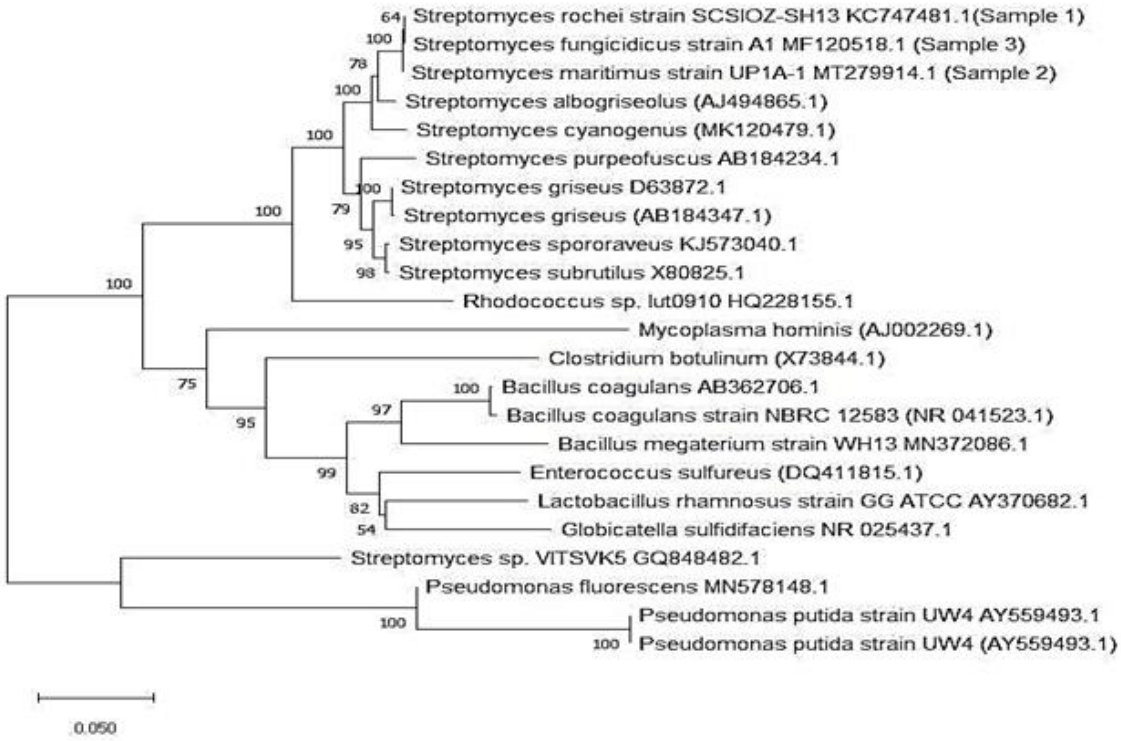

Fig. 2. Phylogenetic tree based on 16S rRNA sequences showing the relationship between isolated *Streptomyces* strains with maximum antibacterial activity and representatives of some other related taxa. Numbers at nodes show percentages of 1000 bootstrap re-samplings, only values above 50% are shown. Bar, 0.050 substitutions per site.

Effect of extracts on cell cycle analysis

The regulation of entrance and advancement through the cell cycles is compromised in cancer cell lines due to altered cell cycle regulatory proteins; as a result, mediators that restrict the proliferation of these cells by controlling these proteins have therapeutic relevance. To test the inhibitory effects of *Streptomyces* extracts on the cell cycle process, cell cycle evaluation was performed. In a dose-dependent manner, both

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cell lines showed a significant increase in the DNA fragmentation as reflected by the increase in the number of the cell's population in sub G1 phase compared to the control cells. In addition, our study showed that extracts induced G2/M cell cycle arrest in HT-29 and HFF cells. For example, exposure of HT-29 cells to 100 µg/mL of Strain-100 extract resulted in a statistically significant increase in the percentage of cells in sub G1 phase from 52.1% (untreated cells) to 72.3% ($p<0.001$). The percentage of cells in sub-G1 phase after 24-hour treatment with Strain-98 extract (150 µg/mL) showed a significant increase ($p \leq 0.001$) (Figure 3). The results follow the study of Pumiputavon *et al.* who investigated cell cycle arrest and apoptosis induction by methanolic leaf extracts of some plants (14). In another similar study, Tan *et al.* found that *Streptomyces sp. MUM256* extract induced cell cycle arrest and apoptosis in colon cancer cells (HCT116) through its bioactive metabolites (21). These findings suggest that the extracts interfere with the cell cycle and inhibit cell proliferation. It is well known that DNA fragmentation is a feature of apoptotic cell death, and an increase in the proportion of subG1 cells is a measure of this DNA fragmentation. In apoptotic cells, Figure 4 shows morphological changes, nuclear condensation, and DNA fragmentation, all of which are consistent with the increasing proportion of subG1 cell populations.

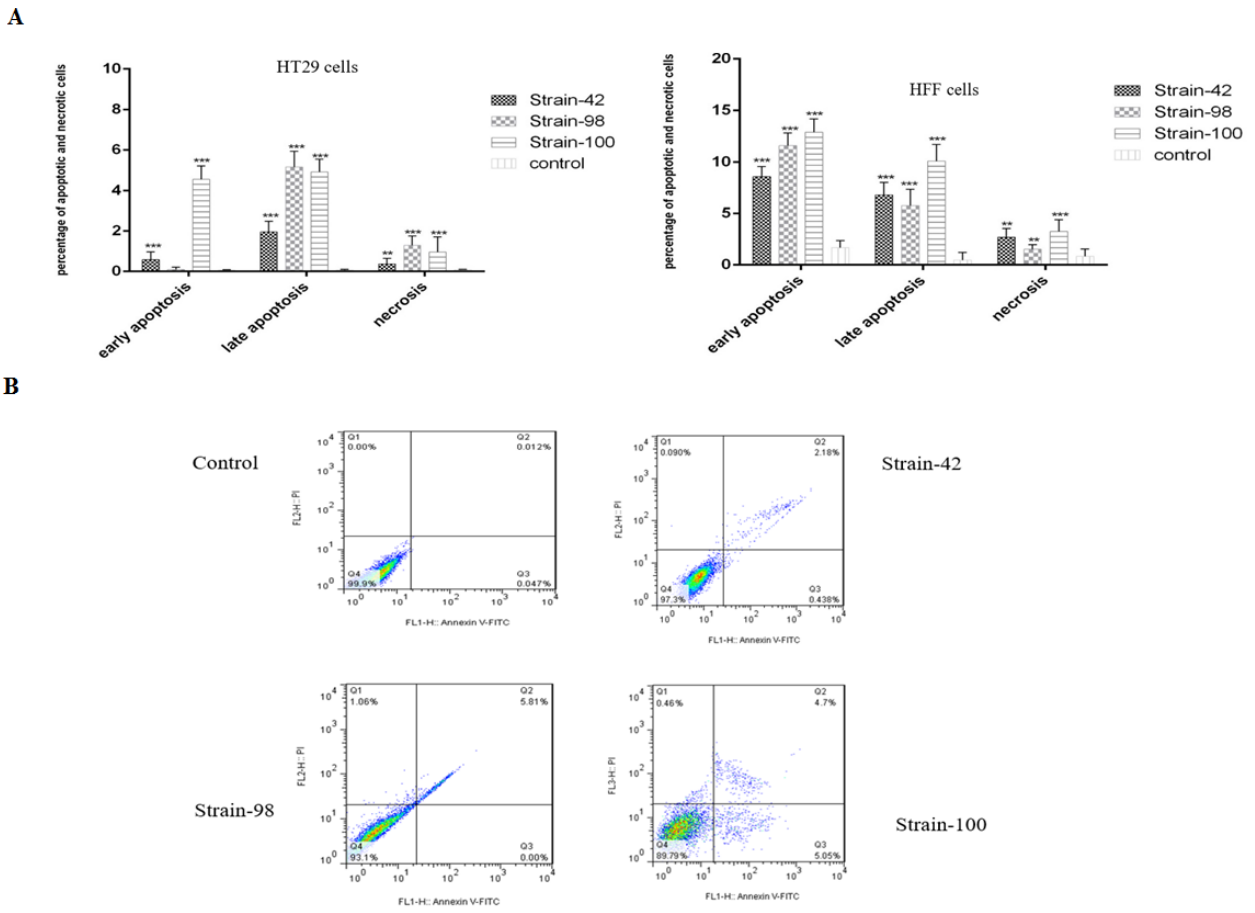

Fig. 3. Representatives of apoptosis evaluation of extracts on cell lines. (A) Dot plots of AnnexinV/FITC-stained cell lines after 48 hours of exposure to extracts (150 µg/mL) of potent *Streptomyces* strains (numbers 42, 98, and 100) in the presence of control. Data are presented as means ± SD. **= $p<0.05$ and ***= $p<0.001$. (B) The apoptosis induction of strain extracts (150 µg/mL) on HT-29 cells was examined by flow cytometry after 48 hours of treatment using Annexin V-FITC /PI staining, and compared to control.

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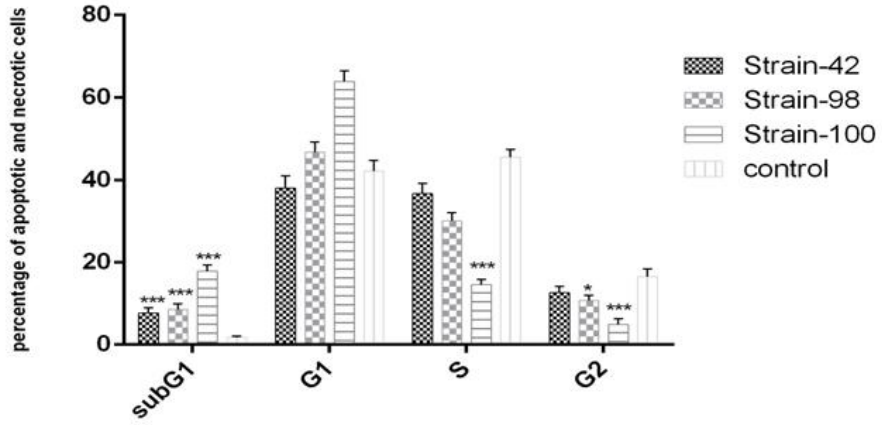

Fig. 4. Comparative dot plots graph of the percentage of necrotic and apoptotic cells in the cell cycle stages due to treatment with *Streptomyces extracts* (100-150 µg/mL) in the presence of the control. * = p <0.05, *** = p <0.001.

Expressions of pro-apoptotic genes

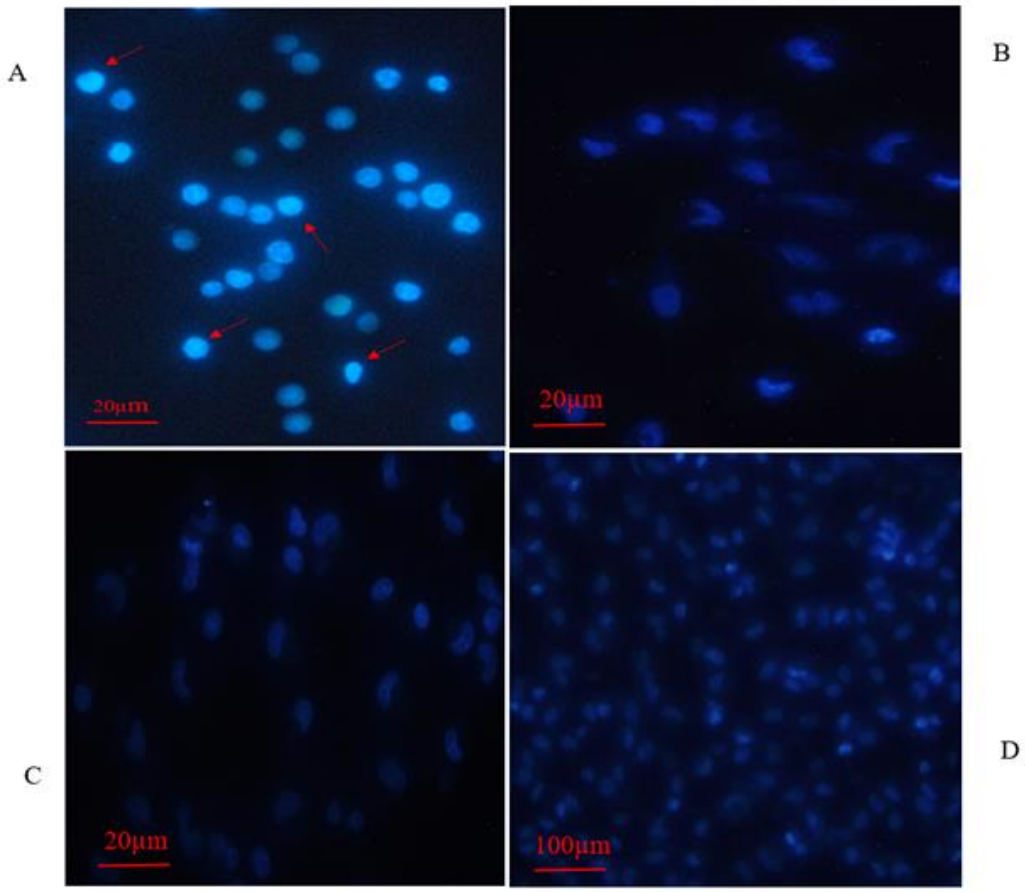

Fig. 5. Apoptotic evaluation of strain 100 (A), strain-98 (B), and strain-42 *Streptomyces* extract (150 µg/mL) on HT-29 cell line, compared to control (D). Nuclear morphology of human colon cancer cell lines (HT-29) stained by DAPI. Comparison of the nuclear morphological properties of untreated (Right) and treated HT-29 cells (Left) under fluorescence microscope. Red arrows point to the nuclear condensation and fragmentation caused by the cytotoxic effect of Strain-100 extract, indicating symptoms of apoptosis.

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The transcription rate of apoptotic genes was analyzed to confirm the flow cytometry results and to determine the mechanism of action of the cytotoxic effects of the extracts on the cells. The qRT-PCR showed that the expression levels of *caspase 8/3*, *p53* and *p21* were significantly upregulated after treatment with the extracts of the strains (Figure 5). Strains-100 induced the strongest effects on the expression of target genes. Overall, the extracts induced the expression of these pro-apoptotic genes to varying degrees, accompanied by cell death, suggesting that the extracts are involved in caspase-dependent cell apoptosis. In addition, this result supports that activation of p21 and *p53* plays a role in *Streptomyces-induced* apoptotic cell death in both cell lines. Previous studies have illustrated that p21 is a transcriptional target of the *p53* protein, and it could be concluded that changes in *p53* upon treatment with *Streptomyces* extracts affect *p21* expression levels. The bcl-2 family is involved in the process of point-of-no-return by regulating mitochondrial transmembrane permeabilization. In this study, *Bax* gene expression levels of cell lines treated with the extract showed no detectable changes. Furthermore, our studies showed that treatment with the extract led to a down-regulation of anti-apoptotic Bcl-2 gene expression.

Discussion

A promising area of cancer research is the investigation of the cytotoxicity of secondary bioactive chemicals produced by Streptomyces in soil against a colon cancer cell line. The Gram-positive bacterium Streptomyces, known to produce large quantities of antibiotics and other bioactive compounds (22), has a wealth of promising anticancer drugs and produces a variety of secondary metabolites that thrive in soil as part of its natural defense system. The aim of recent research is to utilize these substances for potential medicinal purposes.

The urgent need for more effective and targeted cancer therapies is the reason for the particular interest in their cytotoxic effect against colorectal cancer cell lines. By isolating and evaluating these secondary metabolites, researchers can find new molecules with potent anticancer properties, opening up new possibilities for therapeutic development (23). Given the increasing studies on the benefits of microorganisms, especially bacteria, and their increasing use in various areas of the pharmaceutical and food industries, it seems that their practical aspects in cancer treatment should also be considered (24). According to World Health Organization statistics, cancer is now the second leading cause of death worldwide after cardiovascular disease. Colorectal cancer is the most common cancer of the gastrointestinal tract. The main cause is not known, but it is the third most common cancer in women after lung and breast cancer in terms of prevalence.

The main risk factors for the occurrence of this cancer are age over 50 years and a family history, followed by diet, obesity, physical inactivity and smoking (25). The need to find new compounds for the production of antimicrobial and anticancer metabolites is growing, as shown by numerous studies in the field of antimicrobial and anticancer drugs. These studies highlight the increasing prevalence of antibiotic resistance, the spread of cancer as one of the leading causes of death worldwide, resistance to chemotherapeutics and the occasionally harmful side effects of this class of drugs. The areas of antimicrobial and anticancer activity have received little attention and there is a need to find new indigenous strains that produce novel metabolites (26). Therefore, this study aims to identify *Streptomyces* bacteria that produce

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secondary metabolites from soil and investigate their antimicrobial and anticancer effects against colorectal cancer cell lines using cytotoxicity assays.

According to the results of the present study, three native strains of *Streptomyces* exhibited a broad spectrum of bactericidal effects on both Gram-positive and Gram-negative bacteria through secondary metabolites. In the HFF and HT29 cell lines, these metabolites significantly ($p<0.05$) and highly significantly ($p<0.001$) increased the expression of the genes Casp8, b-actin, Bax, Bcl2, p21, p53 and Casp3. The flow cytometric results of the apoptosis assay showed that the sample extracts induced late apoptosis and necrosis ($p<0.001$) in both cell lines and early apoptosis ($p<0.001$) in the HT29 cell line. The effect of these extracts on the nuclei of the cell lines was validated by DAPI staining, which showed a dose-dependent inhibition of cell growth and induction of cell death. In the current study, the natural strain of Pseudomonas UW4 metabolites was used to selectively inhibit the development of breast cancer cell lines. The present study investigated the antibacterial efficacy of the metabolites of the indigenous strain of Pseudomonas UW4 against a variety of harmful microorganisms. The information gathered demonstrated that Pseudomonas UW4 can produce antibiotic compounds that are effective against *Staphylococcus aureus*. Treatment with the metabolite indicated that the biological potential of the cells decreased with increasing concentration in a dose- and time-dependent manner.

Consequently, the highest effect was associated with a concentration of 20 mg/ml and occurred 72 hours after cell treatment ($P<0.01$), while metabolites at all concentrations had no detectable effects on conventional fibroblast cells (27). This result is consistent with that of the present study on the antibacterial effect of secondary metabolites on common bacteria. Additionally, the information on the induction of cell death in cancer cell lines agreed with the conclusions of the ongoing study.

Moosavi et al. (2012) investigated the effect of secondary metabolites on apoptosis. In their study, they investigated ether-soluble metabolites of an indigenous Iranian bacterium called *Streptomyces* sp. ABRIINW 111. The isolation and anticancer effects of the metabolites were studied using the chronic myeloid leukemia cell line K562. The data showed that ether-soluble metabolites inhibited the concentration- and time-dependent growth of K562 cells. In addition, these metabolites caused a significant decrease in the viability of K562 cells ($P<0.05$). It was also announced that the results of light microscopy observations and DNA fragmentation assay indicated the induction of apoptosis in K562 cells. Their conclusion represented that considering the defects in the apoptosis process in cancer cells and the presence of drug resistance in these cells, the identification of new apoptosis-inducing compounds such as ether-soluble metabolites may be helpful for further studies in the field of cancer treatment (28). The ongoing study also proves that the secondary metabolites of native *Streptomyces* can induce apoptosis in cancer cell lines by increasing programmed cell death induction genes.

In 2017, Zhang et al. studied the antitumor effect of Rhodococcus Lut0910 isolated from polluted soil on two types of liver cancer cells (HepG2) and uterine cancer (Hela). Both in vitro and in vivo, the extract of the bacterium showed antitumor effects on these two cell lines.

The application of *Rhodococcus* Lut0910 extract to the investigated cancer cells led to a dose-dependent inhibition of cell division. In addition, mice with certain tumors showed less tumor development when the bacterial extract was adminestired orally compared to the control group (29). These results are

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consistent with the conclusions of the current study on the protective properties of the secondary metabolites of soil bacteria against cancer. Mandale et al. (2017) investigated the antibacterial effect of bacteria isolated from soil on the three human pathogens *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Their results revealed that only 3 of the 16 isolates isolated *Bacillus megaterium*, *Pseudomonas fluorescens* and *Globicitella sulfidifasein* had antimicrobial activity on the three human pathogens mentioned and the best antibacterial effect of these three isolates was on Staphylococcus aureus (30).

In contrast to our study, Rodriguez's study examined the occurrence of cytotoxic properties along with the change in bacterial growth conditions and recorded the optimal values for each variable.

They cleared that *Streptomyces psammoticus* bacteria had IC50 values of 35.53 against breast cancer cells, and these values in Ravikumar's study for *Acinetobacter* strains against breast cancer cell lines were 32.79 (31). In 2018, Karpinski et al. reported that the antiproliferative peptide Entap, produced by enterococcal bacteria exerts antiproliferative properties on the cell line (22Rv1) by arresting the cycle. The growth and proliferation of cancer cells occurs in the G1 phase of the cell cycle, as does the induction of apoptosis and autophagy (32). In a study conducted by Gislin et al. in 2018, the antibacterial effect of Gram-positive bacteria isolated from 10 different regions in Kochi, India, on 6 types of human pathogenic bacteria was investigated. Their results indicated that the isolates isolated from the soil had a positive and significant antibacterial effect on two human pathogenic bacteria, Enterococcus and Staphylococcus aureus. These researchers concluded that the microbial isolates isolated in the present study can be used commercially for the production of antibiotics after purification and standardization (33).

In this study of bioactive Streptomyces compounds isolated from soil and their anticolorectal and antibacterial properties, several possible limitations should be considered.

Geographical limitations: Only soil samples were collected from Kerman, Iran. The diversity of Streptomyces species with potential bioactive properties that may exist in other areas may not have been fully captured by this limited sampling. A wider range of Streptomyces strains and possibly more effective bioactive chemicals could be obtained through a broader geographic survey. Cell lines used: HFF cells and the colon cancer cell line HT29 are the main objects of investigation. These models are useful, but they do not adequately reflect the complexity of cancer biology in vivo. Additional primary cells and cancer cell lines could provide a more comprehensive understanding of the efficacy and selectivity of Streptomyces extracts. Specificity and mechanism of action: Although the study shows that Streptomyces extracts can stop cell growth and induce apoptosis, the exact biochemical pathways behind these effects are still unknown. In-depth mechanistic studies are needed to understand how these extracts interact with cellular targets and pathways. It would be important to precisely determine the bioactive compounds and their molecular targets that cause the observed effects.

Possible cytotoxicity and adverse effects: Research suggests lower cytotoxicity to healthy cells as opposed to cancer cells. However, more comprehensive toxicity studies are needed to ensure the safety of these extracts. A more comprehensive understanding of the therapeutic window and potential adverse effects would be gained by conducting long-term toxicity studies and evaluating the effects on larger numbers of normal cells.

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If these shortcomings were addressed by further research, the results could become more reliable and applicable, opening the door to the potential therapeutic use of bioactive chemicals generated from Streptomyces in the treatment of bacterial infections and colorectal cancer.

In conclusion, the results of the present study revealed that the extracts of *Streptomyces rochei*, *Streptomyces fungicidicus* and *Streptomyces maritimus* indicated a significant antimicrobial activity and in vitro inhibition of proliferation ability of HT-29 and HFF cell lines. Morphological changes observed in the cells treated with the extracts represent the occurrence of cell death by apoptosis mediated by the activation of caspase and *p53*/*p21* proteins. The current study provides preliminary results suggesting that soil-isolated *Streptomyces* strains represents a valuable source of potentially novel bioactive secondary metabolites against colon cancer that act through the activation of proapoptotic proteins. Further studies are needed to understand the antimicrobial properties of *Streptomyces*.

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