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Original Article

Secondary Metabolites of Soil Actinomycetes, UTMC 676 and UTMC 919, Induces Apoptosis in Human Non-Small-Cell Lung Cancer Cell Line

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Abstract

Background & Aims: Bacterial metabolites are extremely rich resources for discovering new compounds with different biological activities. Metabolites of actinomycetes have significant potential for the production of anticancer compounds. The purpose of this research is to investigate the effects of two secondary metabolites of soil actinomycetes, UTMC 676 and UTMC 919, on apoptosis induction and their related genes in the human non-small cell lung carcinoma cell line, A549.

Materials & Methods: The crude extracts of UTMC 676 and UTMC 919 were prepared from the collection of biological compounds of Tehran University. After cell treatment with UTMC 676 and UTMC 919, cell cytotoxicity, apoptosis, and mRNA expression were measured using MTT, flow cytometry, and q-RT-PCR methods. Doxorubicin was utilized as a positive control.

Results: The MTT results showed induction of cytotoxicity by UTMC 676, UTMC 919, and doxorubicin in A549 cells in a concentration-dependent manner. After 48 hours of treatment, both UTMC 676 and UTMC 919 induced apoptosis in the A549 cell line. However, the apoptotic effect of UTMC 676 was more than doxorubicin. The q-RT-PCR data exhibited that the expression of apoptosis-related genes was enhanced in the treated group compared to the untreated group.

Conclusion: These results suggest that the crude extract of UTMC 676 was able to induce apoptosis in A549 cells and could be a very promising source having therapeutic potential against lung cancer cell lines.

***Keywords*:** Lung cancer, Apoptosis, Soil actinomycetes, Doxorubicin

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Introduction

Lung cancer is the most common type of cancer in both genders, and according to International Agency for Research on Cancer (IARC), it accounts for 18.4% of total cancer-related deaths in 2018; about 1.8 million deaths estimated in 2018 occurred due to lung cancer (1). In Iran, lung cancer is the second and third leading cause of cancer deaths for men and women, respectively (2). There are several risk factors development of lung cancer, and cigarette smoking can be the most important. Histologically, non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC) are two common types of lung cancer, accounting for 85% and 15-20% (depending on the

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region) of all lung cancers, respectively (3). According to the world health organization (WHO) classification, NSCLC is classified into three large subtypes: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma (4). Depending on the severity of the disease, patients with lung cancer receive certain treatments from surgery to chemotherapy, radiotherapy, and targeted therapy. Surgery remains the first treatment for the early stages of NSCLCs. Because the onset of lung cancer is often asymptomatic and the patients are diagnosed in advanced stages, surgery is not useful (5-7). Chemotherapy interventions and radiotherapy are promising methods to control the progression of lung cancer (8). The efficacy of chemotherapy has been confirmed in various stages of NSCLC due to the third generation introduction of cytotoxic drugs such as paclitaxel, docetaxel, and gemcitabine. These compounds often induce apoptosis and suppress metastasis, inhibiting cancer growth and development (9, 10). Many studies have suggested that chemotherapeutic drugs-caused cell death may involve mitochondrial pathway-derived apoptosis (11-13). Specifically, some anti-cancer agents or their metabolites led to increase the levels of pro-apoptotic genes such as Bax and decrease the expression of the anti-apoptotic Bcl2 gene. Bax causes the mitochondrial membrane permeability, resulting in the cytochrome c release from the mitochondria (14). For instance, the upregulation of Bax and mitochondrial release of cytochrome c has been observed in a variety of cancer cells treated with 5-fluorouracil and cisplatin (15-17). However, apoptosis plays a major role in cancer cell death and is therefore targeted in treating these diseases (18, 19). However, drug resistance and toxic effects of chemotherapy drugs on healthy cells have been reported in patients with lung cancer (20, 21).

Therefore, it seems essential to find new treatment strategies to improve the treatment of lung cancer. Today, natural compounds and their derivatives play an important role in the clinical treatment of various types of cancers, making up about 63% of commercially available drugs (19, 22, 23). Some compounds such as vincristine, etoposide, and paclitaxel are examples of plant-derived anticancer drugs, and compounds such as actinomycin D, mitomycin C, bleomycin, doxorubicin, and L-asparaginase are compounds derived from microorganisms (23-25). About 23,000 secondary metabolites have been discovered from microorganisms, and approximately about 10,000 species (about 45%) have been extracted from actinomycetes (26, 27). Actinomycetes belong to the *Actinoabateria* category and are the most valuable prokaryotes responsible for antibiotics production and antitumor metabolites (28, 29). With the great diversity of actinomycetes and their wide applications in various industries, especially pharmacy, researchers have discovered new functional metabolites (26, 30, 31). Due to the increasing use of actinomycetes in the production of various compounds, especially anticancer compounds, for the first time in this study, two secondary metabolites of actinomycetes, UTMC 676 and UTMC 919, were used to evaluate apoptosis in human lung cancer cell line. To achieve this goal, the toxicity of secondary metabolites on the A549 cancer cell line is evaluated first. Then the effective dose of these extracts was used to induce apoptosis in this cell line and then quantify the expression of genes related to apoptosis.

Materials & Methods

The findings of the present study were obtained from a research project of the Department of Microbial Biotechnology University of Tehran through the following approved code: 1398/17.

Human A549 cell culture:

In this experimental study, the cell line, A549, was obtained from the Iranian Biological Resource Center.

The A549 cell line is a human pulmonary epithelial cell line, which morphologically as a single layer on the bottom of the culture flask. Briefly, A549 cells were cultured in a T-75 culture flask containing Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with FBS (Gibco, USA) and penicillin/streptomycin (Gibco, USA), and then maintained at 37 °C in a humidified atmosphere.

Preparation of microbial extract and treatment:

The crude extract of UTMC 919 and UTMC 676 strains was obtained from the biological compounds of Tehran University. The treatments of A549 cells were performed by exposing cells to different concentrations of UTMC 919 (6, 12, 24, and 48 ug/ml) and UTMC 676 (6, 12, 24, and 48 ug/ml) as well as doxorubicin (Sigma, USA) (0.125, 0.25, 0.5, and 1 µM) in serum-free DMEM for 48 hours. After treatments, A549 cells were trypsinized and collected for subsequent analyses.

Cell viability assessment:

The MTT assay (Sigma, USA) is a colorimetric assay for evaluating cell metabolic activity as a cell viability indicator. 10^4 A549 cells per well were first seeded into a 96-well plate containing 100 µl of DMEM culture medium to perform this test. Subsequently, cells were treated with different concentrations of UTMC 863, UTMC 676, and doxorubicin. In the next step, after 48 hours of treatment, 10 µl of MTT solution was added to all wells to investigate the toxicity of the metabolites and then incubated in an incubator at 37 ° C for 4 hours. After incubation time, the culture medium was completely discarded, and dimethyl sulfoxide (DMSO) (Sigma, USA) was added to dissolve the formazan crystals. Then the adsorption of each well was determined at 560 nm by a microplate reader.

Evaluation of the morphology of A549 cells:

In brief, A549 cells (8×10^4 cells/well) were cultured into a 6-well plate and then exposed to UTMC 676, UTMC 919, and doxorubicin for 48 hours. Subsequently, the morphological changes of A549 cells were evaluated using light microscopy (× 10 magnification) (Jenamel, Germany).

Apoptosis assay:

An apoptosis assay typically quantifies the percentage of dead cells related to membrane alterations, DNA fragmentation, and mitochondrial damage. Apoptosis detection of A549 was determined using Apoptosis Detection Kit (BD Biosciences, USA). A549 cells (8×10^4 cells/well) were plated into a 48-well plate for 24 hours and then treated with UTMC 676, UTMC 919, and doxorubicin for 48 hours. After incubation time, A549 cells were harvested and stained with annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) for 15 minutes at room temperature in the dark. The apoptosis rate of each sample was determined using a flow cytometer.

Quantitation of mRNA expression:

The real-time quantification PCR (qRT-PCR) technique has become the main tool for quantification of the RNA. In the current project, the expression level of Bax, p21, caspase-7 (Casp7), p53, and retinoblastoma (Rb) genes was measured using qRT-PCR technique. After cell treatment, total RNA was isolated from treated cells using a total RNA isolation kit (CinnaGen, Iran) based on the manufacturer's protocol. According to the manufacturer's instruction, cDNA was synthesized from isolated RNA by First Strand cDNA Synthesis Kit (TAKARA, Japan). At the final step, qRT-PCR was performed on 7500 Fast Real-Time PCR Detection System (Applied Biosystem, USA) using SYBR Premix Ex Taq TM Master mix (TAKARA, Japan) and specific primers (Table 1). The amplification of each PCR reaction begins by an initial denaturation at 95°C for 2 minutes, followed by 40 cycles containing 5 seconds of denaturation at 95°C and 30 seconds of

annealing/extension at 60 °C. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was employed as an internal control for the normalization of gene expression. The relative expression of the data was calculated by the $2^{-\Delta\Delta Ct}$ method using REST software.

Table 1. The specific sequences of primers used in this study

Gene product	Primer sequences	Product size(bp)
GAPDH	Sense 5′- CCTCAAGATCATCAGCAATG-3′ Antisense 5′- CATCACGCCACAGTTTCC-3′	90
Bax	Sense 5′-CAAACTGGTGCTCAAGGC-3′ Antisense 5′-CACAAAGATGGTCACGGTC-3′	178
Caspase-7	Sense 5′-CACGGTTCCAGGCTATTAC-3′ Antisense 5′-GGCAACTCTGTCATTCACC-3′	139
p21	Sense 5′-CCAGCATGACAGATTTCTACC -3′ Antisense 5′-AGACACACAAACTGAGACTAAGG-3′	150
P53	Sense 5′-GGAGTATTTGGATGACAGAAAC-3′ Antisense 5′-GATTACCACTGGAGTCTTC-3′	181
Rb	Sense 5′-AATCATTCGGGACTTCTG-3′ Antisense 5′-ACTTCCATCTGCTTCATC-3′	154

Statistical analysis:

The results were expressed as mean ± SD (standard deviation), and all statistical analyses were performed using SPSS software version 19 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism software version 8.0 (GraphPad Software, San Diego, CA, USA). In this study, one-way analysis of variance (ANOVA) was used to analyze statistical differences between studied groups, and data with $p<0.05$ were considered as significant.

Results

The effect of actinomycetes secondary metabolites on cell viability:

As shown in Figure 1, the findings of the MTT assay revealed that the crude extracts of UTMC 676, UTMC 919, and doxorubicin could markedly alleviate the A549 cell viability after 48 hours of treatment in a concentration-dependent manner. Both extracts with 24µg/ml concentration could suppress approximately

50% of A549 cell viability (Figure 1). According to the results of the MTT test, the effective concentration of UTMC 676, UTMC 919, and doxorubicin was 48 µg/ml, 48 µg/ml, and 1 µM for further analyses.

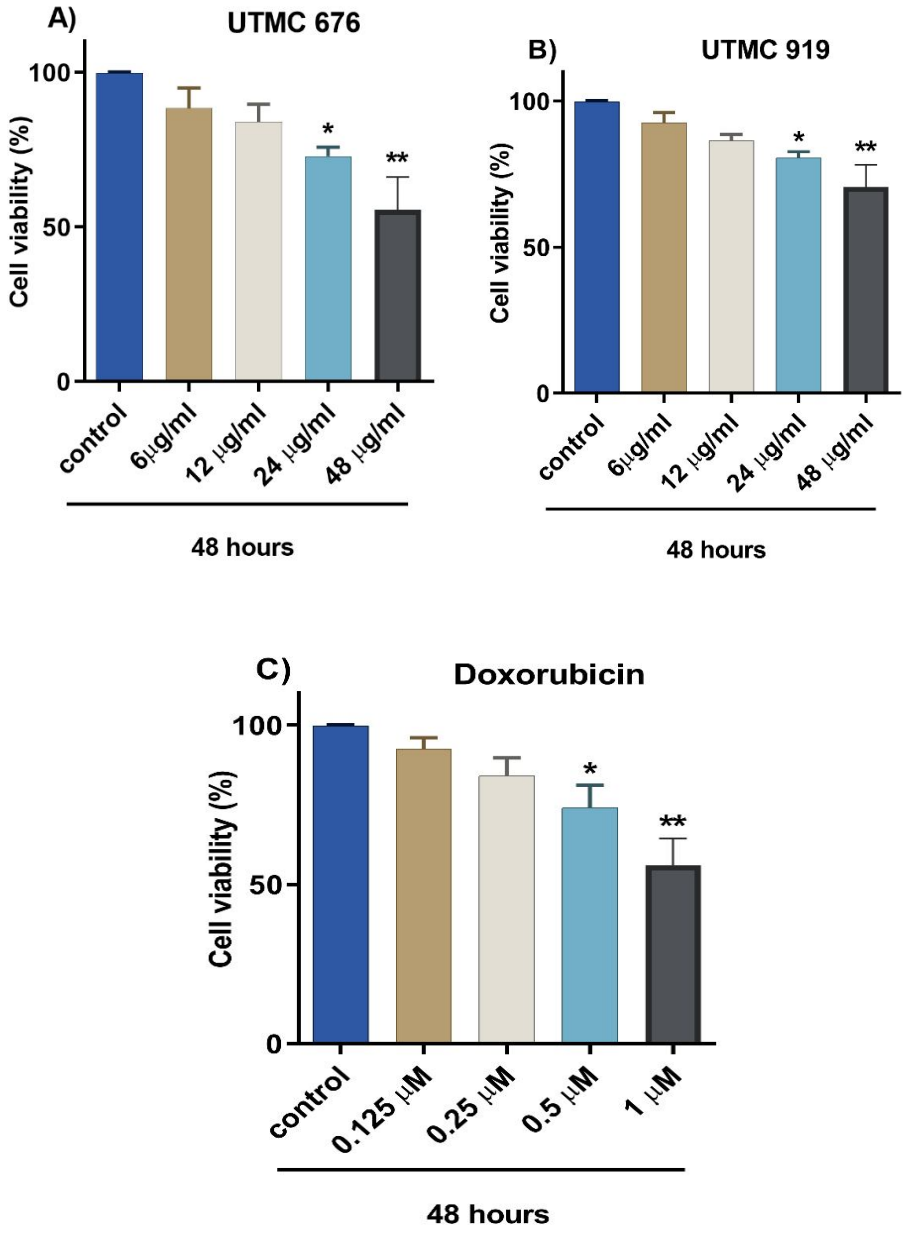

Fig 1. The effect of secondary metabolites of A) UTMC 676, B) UTMC 919, and C) doxorubicin on the viability of the cells of A549. Doxorubicin is regarded as a positive control. The cell viability was monitored using MTT assay and the analyzed results were reported as mean ± SD (standard deviation). $^{*}P<0.05$ and $^{**}P<0.01$ were considered statistically significant.

The effect of actinomycetes secondary metabolites on morphological changes of A549 cells:

The photographs of light microscopy exhibited that treatment of A549 cells with UTMC 919, UTMC 676, and doxorubicin caused changes in the cell shape and morphology. As depicted in Figure 2, untreated cells revealed mainly normal morphologies, whereas the A549 cells treated with UTMC 919, UTMC 676, and doxorubicin, showed a morphological change and a marked increase in cell death.

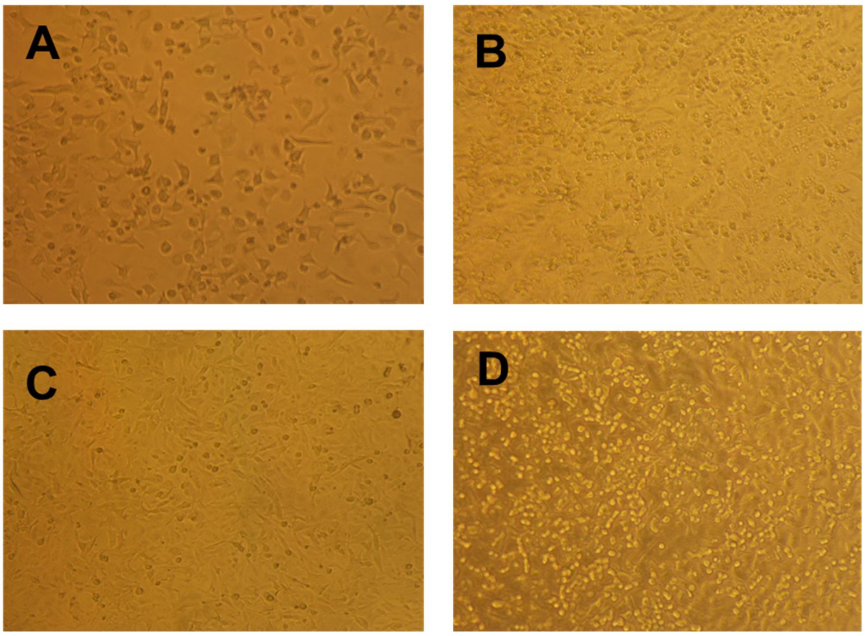

Fig 2. Morphological changes of A549 cells were exposed to A) DMSO (negative control), B) doxorubicin (positive control), C) the crude extract of UTMC 676, and D) the crude extract of UTMC 919. Treated A549 cells morphologically showed evidence of cell death compared with the negative control cells. Morphological changes of cells were visualized by light microscopy (× 10 magnification).

The Effect of Actinomycetes Secondary Metabolites on Apoptosis Rate:

Findings from the flow cytometry show that the crude extract of UTMC 919 and UTMC 676 have same effect of doxorubicin in inducing apoptosis in the A549 cell line (Table 2, Figure 3). As shown in Table 2, the percentage of apoptotic cells and necrotic cells in the cells treated with UTMC 676 was 99.2% and 0.8%, respectively. Simultaneously, the apoptotic and necrotic cells resulting from doxorubicin treatment were about 38.35% and 37.45%, respectively. Furthermore, the apoptotic rate in UTMC 676 group was notably higher than those exposed to doxorubicin. It should also be noted that the percentage of necrotic cells UTMC 919 extract-treated cells was similar to doxorubicin-treated cells. However, the percentage of apoptotic cells in UTMC 919-treated cells (10.53%) was lower than that of cells treated with doxorubicin (38.35%) (Figure 3).

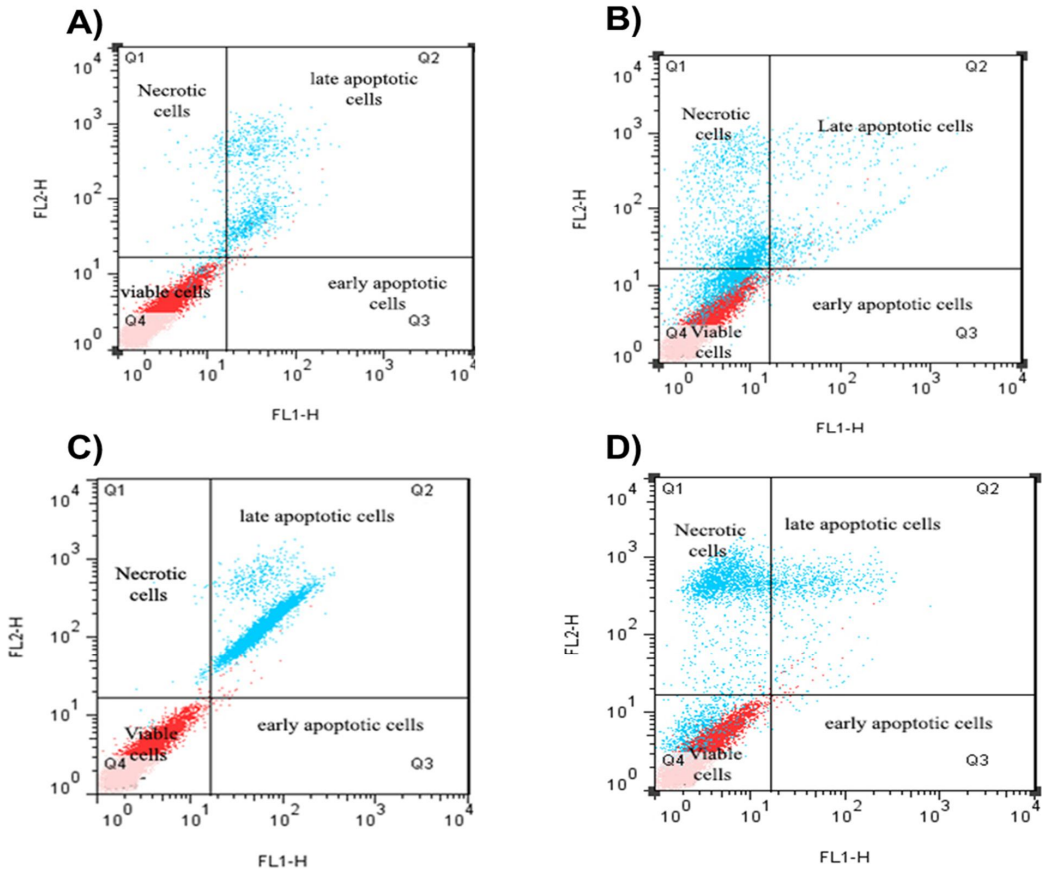

Fig 3. The effect of secondary metabolites of A) UTMC 676 (48 µg/ml), B) UTMC 919 (48 µg/ml), and C) doxorubicin on the apoptosis rate of A549 cells. The cells were exposed to UTMC 676, UTMC 919, and doxorubicin for 48 hours and then the cell apoptosis rate was detected using flow cytometry technique. Doxorubicin (1 µM) is considered a positive control.

Table 2. The effect of UTMC 676, UTMC 919, and doxorubicin on apoptosis of A549 cells

Group	Flow cytometry (%)			
	Early apoptosis	Late apoptosis	Necrosis	alive
Control	0.178±0.007	2.905±0.417	21.5±3.111	75.4±2.687
UTMC 676	0.00±0.00	77.3±0.141*	17.7±0.848	4.98±1.08*
UTMC 919	0.293 ± 0.008	99.2 ± 0.141	0.727 ± 0.067	0.07 ± 0.1
Doxorubicin	1.755±0.2*	38.35±8.697*	37.45±8.838	22.45±0.07*

All variables are reported at mean±S.D. *P<0.05 was considered statistically significant.

The Effect of Actinomycetes Secondary Metabolites on apoptosis Gene Expression:

The quantitative analysis of Bax, Rb, p21, p53, and Casp7 apoptotic genes in A549 cells treated with UTMC 676, UTMC 919, and doxorubicin was shown in Figure 4. The results of the q-RT-PCR analysis demonstrated that Bax, Rb, p21, p53, and Casp7 gene expression were up-regulated in the treated cells, compared with untreated cells. Figure 4 shows that the mRNA expression of Bax, Rb, p21, and Casp7 genes in A549 cells treated with UTMC 676 was higher than it in the cells treated with doxorubicin; however, the expression of the p53 gene is almost similar (Figure 4).

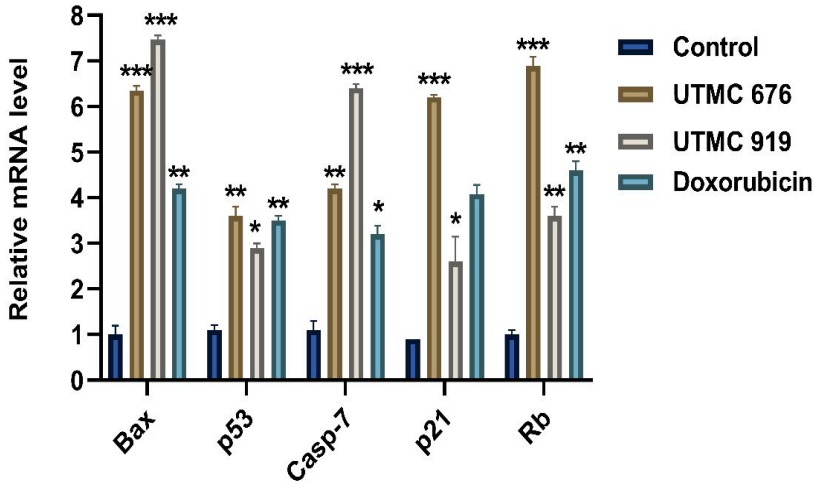

Fig 4. Relative mRNA expression of Bax, Rb, p21, p53, and Casp7 genes in A549 cells exposed to DMSO (control), the crude extract of UTMC 676, the crude extract of UTMC 919, and doxorubicin (positive control). The cells were exposed to UTMC 676, UTMC 919, and doxorubicin for 48 hours; then the transcript levels of Bax, Rb, p21, p53, and Casp7 genes were quantified using q-RT-PCR. The analyzed findings were presented as mean ± SD and $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.01$ were considered statistically significant.

Discussion

Lung cancer incidence and its related death have been a serious concern to human societies (32, 33). Given the drug resistance and side effects of chemotherapy drugs, it is considered necessary to identify new anticancer compounds (34). Many antitumor compounds are natural products or their derivatives and are mainly produced by microorganisms (35, 36). Actinomycetes produce many natural compounds with diverse biological activities, such as anticancer properties (37, 38). Since 1950, many soil actinomycete metabolites have been studied for antibiotic, anticancer, and antitumor properties. Food and Drug Administration (FDA) approved some microbial metabolites, such as doxorubicin, mitomycin C, pentostatin, bleomycin, actinomycin, and anthracycline, which are used clinically (39-41). Actinomycin D is one of the first microbial metabolites used to treat cancer. The use of anthracyclines, doxorubicin, and mitomycin C has also been somewhat

successful in treating human lung cancer (42-45). Gao and its colleagues studied the BM-17 strain of marine actinomycetes. They reported that this strain had toxic effects against A549, HepG2, HCT-116, and COC1 cells (46). Besides, anticancer properties have been reported in another group of marine actinomycetes isolated from deep-sea sediment. In this screening, several actinomycete strains have an inhibitory effect on cell growth in breast cancer cell lines MCF-7 and MDA-MB-231 (47). In our research, we examined for the first time the antitumor activities of two secondary metabolites of soil actinomycetes, UTMC 676 and UTNC 919, on lung cancer cells. UTMC 676 strain had 99.86% similarity to *Streptomyces aureoverticillatus*, and UTMC 919 strain was 99.29% similar to *Kribbella sancticallisti*, but according to several articles, there is no report on the production of anticancer compounds in these strains. Like many strains of actinomycetes, our MTT results also showed an inhibitory effect on the growth of A549 cells.

One of the antitumor actions of actinomycetes usually occurred through induction of apoptosis in tumor cells. Our findings showed an increase in apoptosis and a decrease in the necrosis of the cells after treatment with UTMC 676 but not UTMC 919. Research by Rambabu et al. showed that the purified compound of Streptomyces. Sp namely Quinostatin induces apoptosis in the MCF-7 carcinoma cell line (48). In another study, the apoptotic activity of migrastatin compound extracted from Streptomyces platensis on HEPG2 cells was revealed (49).

The alteration in the p53 expression has been reported in various cancers, including lung, colon, and breast cancers (50, 51). Balachandran and his colleagues examined antitumor effects of flavonoids extracted from Streptomyces sp. on A549 cells. Their data showed that the expression of p53 and caspase 3 genes was increased after treatment of A549 cells with the desired flavonoid, indicating apoptosis induction of cancer cells (52). Many observations confirmed that the p53 gene directly affects mitochondrial function during apoptotic processes. Some apoptotic stimuli have been exhibited to induce rapid transfer of p53 to the mitochondrial outer membrane. P53 induces pro-apoptotic genes such as Bax and reduces the expression of the anti-apoptotic Bcl2 gene. Bax leads to increase the permeability of the mitochondrial membrane, which causes to subsequentially release of cytochrome c from the mitochondria, and by acting on apoptotic protease activating factor 1 (Apaf-1) which leads to apoptosome formation and caspase cascade activity (53-55). Also, p53 as a tumor suppressor gene can induce the expression of the p21 gene as its downstream gene, which then suppresses RB by inhibiting cyclin E and CDK2. RB acts as a tumor suppressor and binds to E2F, causing blocking of the cell cycle progression from stage G1 to stage S, and therefore, cell cycle (56, 57). Zhang et al. found that marine Streptomyces sp. derived antimycin analogs alleviated E6/E7 levels and promoted apoptosis in HeLa cells by the reactivation of the p53 and RB (58). In the present study, consistent with the results of other studies on different strains of actinomycetes, the study of the expression profile of apoptotic genes also shows an increase in gene expression and induction of apoptosis in A549 cells.

Using crude extract was one of the limitations of this study. The effective component has not been identified yet, and further investigations are still in progress. Other genes involved in apoptosis, particularly the extrinsic pathway of apoptosis, were not studied due to financial issues.

Conclusion

The use of potential substances that lead to the induction of apoptosis is a very good idea to treat lung cancer. For the first time, the current study reported that a secondary metabolite of soil actinomycetes, crude extracts of UTMC 676, potentially induce apoptosis and

up-regulated apoptosis-related genes in the human lung cancer cells.

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Conflict of interests

The authors declare that there is no conflict of interests associated with this work.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68(6):394-424.
2. Khazaei S, Mansori K, Soheylizad M, Gholamaliee B, Khosravi Shadmani F, Khazaei Z, et al. Epidemiology of lung cancer in Iran: sex difference and geographical distribution. Middle East J Cancer 2017;8(4):223-8.
3. Duma N, Santana-Davila R, Molina JR, editors. Non–small cell lung cancer: epidemiology, screening, diagnosis, and treatment. Mayo Clin Proc; 2019: Elsevier.
4. Zappa C, Mousa SA. Non-small cell lung cancer: current treatment and future advances. Transl Lung Cancer Res 2016;5(3):288.
5. Horn L, Mansfield AS, Szczęsna A, Havel L, Krzakowski M, Hochmair MJ, et al. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. N Engl J Med 2018;379(23):2220-9.
6. Rosell R, Karachaliou N. Optimizing lung cancer treatment approaches. Nat Rev Clin Oncol 2015;12(2):75-6.
7. Panji M, Behmard V, Zare Z, Malekpour M, Nejadbiglari H, Yavari S, et al. Suppressing effects of green tea extract and Epigallocatechin-3-gallate (EGCG) on TGF-β-induced Epithelial-to-mesenchymal transition via ROS/Smad signaling in human cervical cancer cells. Gene 2021;794:145774.
8. Reck M, Rabe KF. Precision diagnosis and treatment for advanced non–small-cell lung cancer. N Engl J Med 2017;377(9):849-61.
9. Liu G, Pei F, Yang F, Li L, Amin AD, Liu S, et al. Role of autophagy and apoptosis in non-small-cell lung cancer. Int J Mol Sci 2017;18(2):367.
10. Abazari O, Divsalar A, Ghobadi R. Inhibitory effects of oxali-Platin as a chemotherapeutic drug on the function and structure of bovine liver catalase. J Biomol Struct Dyn 2020;38(2):609-15.
11. Liu L, Fan J, Ai G, Liu J, Luo N, Li C, et al. Berberine in combination with cisplatin induces necroptosis and apoptosis in ovarian cancer cells. Biol Res 2019;52(1):1-14.
12. Croce CM, Reed JC. Finally, an apoptosis-targeting therapeutic for cancer. Cancer Res 2016;76(20):5914-20.
13. Fattah A, Morovati A, Niknam Z, Mashouri L, Asadi A, Rizi ST, et al. The synergistic combination of cisplatin and piperine induces apoptosis in MCF-7 cell line. Iran J Public Health 2021;50(5):1037.
14. Carneiro BA, El-Deiry WS. Targeting apoptosis in cancer therapy. Nat Rev Clin Oncol 2020;17(7):395-417.
15. Li W, Shi Y, Wang R, Pan L, Ma L, Jin F. Resveratrol promotes the sensitivity of small-cell lung cancer H446 cells to cisplatin by regulating intrinsic apoptosis. Int J Oncol 2018;53(5):2123-30.
16. Handali S, Moghimipour E, Rezaei M, Ramezani Z, Kouchak M, Amini M, et al. A novel 5-Fluorouracil targeted delivery to colon cancer using folic acid

conjugated liposomes. Biomed Pharmacother 2018;108:1259-73.

17. Dai XY, Zhou BF, Xie YY, Lou J, Li KQ. Bufalin and 5-fluorouracil synergistically induce apoptosis in colorectal cancer cells. Oncol Lett 2018;15(5):8019-26.
18. Paul I, Jones JM. Apoptosis block as a barrier to effective therapy in non small cell lung cancer. World J Clin Oncol 2014;5(4):588.
19. Maleki N, Yavari N, Ebrahimi M, Faiz AF, Ravesh RK, Sharbati A, et al. Silibinin exerts anti-cancer activity on human ovarian cancer cells by increasing apoptosis and inhibiting epithelial-mesenchymal transition (EMT). Gene 2022;823:146275.
20. Lim Z-F, Ma PC. Emerging insights of tumor heterogeneity and drug resistance mechanisms in lung cancer targeted therapy. J Hematol Oncol 2019;12(1):1-18.
21. Asadi A, Nezhad DY, Javazm AR, Khanicheragh P, Mashouri L, Shakeri F, et al. In Vitro Effects of Curcumin on Transforming Growth Factor-β-mediated Non-Smad Signaling Pathway, Oxidative Stress, and Pro-inflammatory Cytokines Production with Human Vascular Smooth Muscle Cells. Iran J Allergy Asthma Immunol 2020:84-93.
22. Cragg GM, Pezzuto JM. Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. Med Princ Pract 2016;25(Suppl. 2):41-59.
23. Panji M, Behmard V, Zare Z, Malekpour M, Nejadbiglari H, Yavari S, et al. Synergistic effects of green tea extract and paclitaxel in the induction of mitochondrial apoptosis in ovarian cancer cell lines. Gene 2021;787:145638.
24. Xiao Q, Zhu W, Feng W, Lee SS, Leung AW, Shen J, et al. A review of resveratrol as a potent chemoprotective and synergistic agent in cancer chemotherapy. Front Pharmacol 2019;9:1534.
25. Roy A, Jauhari N, Bharadvaja N. Medicinal plants as a potential source of chemopreventive agents. anticancer plants: Natural products and biotechnological implements: Springer; 2018. p. 109-39.
26. Bernardi DI, das Chagas FO, Monteiro AF, Dos Santos GF, de Souza Berlinck RG. Secondary metabolites of endophytic Actinomycetes: isolation, synthesis, biosynthesis, and biological activities. Prog Chem Org Nat Prod 2019;108:207-96.
27. Abbasi M, Mousavi MJ, Jamalzehi S, Alimohammadi R, Bezvan MH, Mohammadi H, et al. Strategies toward rheumatoid arthritis therapy; the old and the new. J Cell Physiol 2019;234(7):10018-31.
28. Jakubiec-Krzesniak K, Rajnisz-Mateusiak A, Guspiel A, Ziemska J, Solecka J. Secondary metabolites of actinomycetes and their antibacterial, antifungal and antiviral properties. Pol J Microbiol 2018;67(3):259.
29. Hozzein WN, Mohany M, Alhawsawi SM, Zaky MY, Al-Rejaie SS, Alkhalifah DHM. Flavonoids from Marine-Derived Actinobacteria as Anticancer Drugs. Curr Pharm Des 2020.
30. Musavi H, Abazari O, Barartabar Z, Kalaki-Jouybari F, Hemmati-Dinarvand M, Esmaeili P, et al. The benefits of Vitamin D in the COVID-19 pandemic: biochemical and immunological mechanisms. Arch Physiol Biochem 2020:1-9.
31. Abazari O, Shafaei Z, Divsalar A, Eslami-Moghadam M, Ghalandari B, Saboury AA, et al. Interaction of the synthesized anticancer compound of the methyl-glycine 1, 10-phenanthroline platinum nitrate with human serum albumin and human hemoglobin proteins by spectroscopy methods and molecular docking. J Iran Chem Soc 2020;17(7):1601-14.
32. Barta JA, Powell CA, Wisnivesky JP. Global epidemiology of lung cancer. Ann Glob Health 2019;85(1).
33. Musavi H, Abazari O, Safaee MS, Variji A, Koohshekan B, Kalaki-Jouybari F, et al. Mechanisms of COVID-19 Entry into the Cell: Potential Therapeutic Approaches Based on Virus Entry Inhibition in COVID-19 Patients with Underlying Diseases. Iran J Allergy Asthma Immunol 2021;20(1):11-23.
34. Terlizzi M, Colarusso C, Pinto A, Sorrentino R. Drug resistance in non-small cell lung Cancer (NSCLC): Impact

of genetic and non-genetic alterations on therapeutic regimen and responsiveness. Pharmacol Ther 2019;202:140-8.

35. Katz L, Baltz RH. Natural product discovery: past, present, and future. J Ind Microbiol Biotechnol 2016;43(2-3):155-76.
36. Maleki N, Ravesh RK, Salehiyeh S, Faiz AF, Ebrahimi M, Sharbati A, et al. Comparative effects of estrogen and silibinin on cardiovascular risk biomarkers in ovariectomized rats. Gene 2022;823:146365.
37. Davies-Bolorunduro OF, Adeleye IA, Akinleye MO, Wang PG. Anticancer potential of metabolic compounds from marine actinomycetes isolated from Lagos Lagoon sediment. J Pharm Anal 2019;9(3):201-8.
38. Fattah A, Asadi A, Shayesteh MRH, Hesari FH, Jamalzehi S, Abbasi M, et al. Fertility and infertility implications in rheumatoid arthritis; state of the art. Inflamm Res 2020;69:721-9.
39. Wang C, Lu Y, Cao S. Antimicrobial compounds from marine actinomycetes. Arch Pharm Res 2020:1-28.
40. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod 2020;83(3):770-803.
41. Barmaki H, Morovati A, Eydivandi Z, Naleshkenani FJ, Saedi S, Musavi H, et al. The Association between Serum Oxidative Stress Indexes and Pathogenesis of Parkinson's Disease in the Northwest of Iran. Iran J Public Health 2021;50(3):606-15.
42. Pinato DJ, Gramenitskaya D, Altmann DM, Boyton RJ, Mullish BH, Marchesi JR, et al. Antibiotic therapy and outcome from immune-checkpoint inhibitors. J Immunother Cancer 2019;7(1):1-8.
43. Deslouches B, Di YP. Antimicrobial peptides with selective antitumor mechanisms: prospect for anticancer applications. Oncotarget 2017;8(28):46635.
44. Musavi H, Tabnak M, Sheini FA, Bezvan MH, Amidi F, Abbasi M. Effect of garlic (Allium sativum) on male fertility: a systematic review. J Herb Med Pharmacol 2018;7(4):306-12.
45. Pourgholi M, Abazari O, Pourgholi L, Ghasemi-Kasman M, Boroumand M. Association between rs3088440 (G> A) polymorphism at 9p21. 3 locus with the occurrence and severity of coronary artery disease in an Iranian population. Mol Biol Rep 2021;48(8):5905-12.
46. Gao X, Lu Y, Xing Y, Ma Y, Lu J, Bao W, et al. A novel anticancer and antifungus phenazine derivative from a marine actinomycete BM-17. Microbiol Res 2012;167(10):616-22.
47. Ravikumar S, Fredimoses M, Gnanadesigan M. Anticancer property of sediment actinomycetes against MCF–7 and MDA–MB–231 cell lines. Asian Pac J Trop Biomed 2012;2(2):92-6.
48. Rambabu V, Suba S, Vijayakumar S. Antimicrobial and antiproliferative prospective of kosinostatin–a secondary metabolite isolated from Streptomyces sp. J Pharm Anal 2015;5(6):378-82.
49. Huang H, Lan X, Wang Y, Tian L, Fang Y, Zhang L, et al. New bioactive derivatives of nonactic acid from the marine Streptomyces griseus derived from the plant Salicornia sp. Phytochem Lett 2015;12:190-5.
50. Aubrey BJ, Kelly GL, Janic A, Herold MJ, Strasser A. How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? Cell Death Differ 2018;25(1):104-13.
51. Dayati P, Rezaei HB, Sharifat N, Kamato D, Little PJ. G protein coupled receptors can transduce signals through carboxy terminal and linker region phosphorylation of Smad transcription factors. Life Sci 2018;199:10-5.
52. Balachandran C, Sangeetha B, Duraipandiyan V, Raj MK, Ignacimuthu S, Al-Dhabi N, et al. A flavonoid isolated from Streptomyces sp.(ERINLG-4) induces apoptosis in human lung cancer A549 cells through p53 and cytochrome c release caspase dependant pathway. Chem Biol Interact 2014;224:24-35.

53. Mohamed R, Dayati P, Mehr RN, Kamato D, Seif F, Babaahmadi-Rezaei H, et al. Transforming growth factor–β1 mediated CHST11 and CHSY1 mRNA expression is ROS dependent in vascular smooth muscle cells. J Cell Commun Signal 2019;13(2):225-33.
54. Pfeffer CM, Singh AT. Apoptosis: a target for anticancer therapy. Int J Mol Sci 2018;19(2):448.
55. Babaahmadi-Rezaei H, Little PJ, Mohamed R, Zadeh GM, Kheirollah A, Mehr RN, et al. Endothelin-1 mediated glycosaminoglycan synthesizing gene expression involves NOX-dependent transactivation of the transforming growth factor-β receptor. Mol Cell Biochem 2022:1-8.
56. Moser J, Miller I, Carter D, Spencer SL. Control of the Restriction Point by Rb and p21. Proc Natl Acad Sci 2018;115(35):E8219-E27.
57. Shahidi M, Moradi A, Dayati P. Zingerone attenuates zearalenone-induced steroidogenesis impairment and apoptosis in TM3 Leydig cell line. Toxicon 2022.
58. Zhang W, Che Q, Tan H, Qi X, Li J, Li D, et al. Marine Streptomyces sp. derived antimycin analogues suppress HeLa cells via depletion HPV E6/E7 mediated by ROS-dependent ubiquitin–proteasome system. Sci Rep 2017;7(1):1-14.

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