In vitro cytotoxic and apoptotic activities of Allium paradoxum (M. Bieb.) G. Don extract on human breast cancer cell line

Naghmeh Gholipour¹, Sakineh Mashjoor*², Malihe Naderi¹, Mohsen Rastgar Pouyani¹⁴, Zeinab Emruzi Tubkanlu¹, Nader Mansour Samaei*³,⁵ & Hamideh Khajeh⁷

¹National Institute of Genetic Engineering and Biotechnology, Department of Medical Genetic, Tehran, Iran;
²University of Hormozgan, Faculty of Marine Science and Technology, Department of Marine biology, Bandar Abbas, Iran;
³Qom branch, Islamic Azad University, Department of Microbiology, Qom, Iran;
⁴Tehran University of Medical Sciences, School of Public Health, Department of Pathobiology, Division of Immunology, Tehran, Iran;
⁵Golestan University of Medical sciences, Faculty of Medicine, Department of Human Genetics, Gorgan, Iran;
⁶Laboratory Sciences Research Center, Gorgan, Iran;
⁷University of Zabol, Faculty of Science, Department of Biology, Zabol, Iran

E-mails: sakynemashjoor@gmail.com, n_samaei@yahoo.com

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Researchers from all pharmaceutical fields are trying to find new drugs from natural origin with less toxicity. In northern Hycranian forests Iran, Allium paradoxum (M. Bieb.) G. Don has traditionally used as food and vegetable. Previously studies reports, this plant has a medicinal potential for anti-oxidant and anti-hemolytic activities. In this regard, we evaluated the anti-tumor activity of hydroalcoholic extract of A. paradoxum (M. Bieb.) G. Don in different concentrations on human breast cancer cell line (MCF-7). MTT assay was performed with MCF-7 cancer cell line and also evaluation of apoptotic effect, Bax and Bcl-2 expression in MCF7 cells were analyzed by real time RT-PCR. The results showed that the A. paradoxum (M. Bieb.) G. Don extracts decrease the viability of MCF-7 cell line in a dose-dependent manner and the most effective concentration of this extracts after 24 h treatment was 100 µM. Apoptosis induction was confirmed by fluorescence microscopy and plant extracts display a pro-apoptotic effect by down-regulated and up-regulated the expression of Bcl-2 and BAX in tumor cells, respectively. In conclusion, the study was confirmed pro-apoptotic and cytotoxicity effect of A. paradoxum (M. Bieb.) G. Don extract against MCF-7 cell lines. Based on being natural, low cost, accessibility, and noteworthy advantages of this product, it seems that A. paradoxum (M. Bieb.) G. Don has a potential source for isolation of novel anticancer agents for a drug.

Keywords: Breast cancer, Allium paradoxum, BAX, BCL-2, MCF-7.

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Cancer is one of the leading causes of death in the world whereas, after many years of research, the treatment of cancer is still a problem. One of these problems is resistance to drugs that are used for the treatment of various cancers. Moreover, common cytotoxic treatments target rapidly dividing cells which include malignant cells as well as normal cells thus leading to significant morbidity and limited clinical approaches⁹. Therefore, today the discovery of new anticancer agents of natural origin is a matter of interest. Last epidemiological studies have shown decreased consumption of plant-based foods in many cancer patients². Therefore, it has therapeutic importance in recognizing compounds in plant extracts with anti-cancer effect.

Breast cancer is a malignant tumor that starts in the cells of the breast. It is the second leading cause of cancer death in women worldwide with greater than 1,300,000 cases and 450,000 deaths each year worldwide³. Breast cancer tumors are classified according to the location of origin into ductal tumors that develop in breast ducts (80 %), lobular tumors that develop inside the lobes (10-15 %) and other subtypes representing less than 10 % of cases diagnosed per year⁴. 12.5 % of women have breast cancer and this rate is growing rapidly in many industrial countries⁵.⁶. Currently, it seems that development in diagnosis and treatment of breast

*Corresponding author
cancer is a critical need. The current conventional treatments such as surgery, chemotherapy, and radiotherapy have many side effects such as drug-resistant cancer, loss of appetite, weakness, mouth soreness, hair loss, weight gain, and resistance to infections, bleeding, and diarrhea. On the other hand, despite using therapeutic strategies there is still a high death rate among cancer patients which indicates failure and disadvantages related to these therapeutic processes. Therefore, scientists are trying to find more effective therapeutic approach against breast cancer and attention is being given to naturally acquired compounds. Suppression of apoptosis plays a major role in breast cancer tumorigenesis. Apoptosis is a tightly regulated process, playing an important role in maintaining the homeostasis of multi-cellular organisms. Dysregulation of apoptosis is a marker of human cancer and contributes to therapeutic resistance that leads to a wide variety of pathological conditions, including AIDS, cardiovascular disease, infectious disease, autoimmunity and neurodegenerative disorders. Resistance to apoptosis is contributing to both the development of cancer and resistance to conventional therapies such as radiation and cytotoxic drugs, which function by activating apoptotic cell death pathways. One of the proto-oncogenes is Bcl-2 which is a regulator protein that regulates cell death (apoptosis), expressed particularly in glandular cells at the end of proliferative phase. Whereas, when the secretary phase initiates, expression of Bcl-2 decreases. Bax, also known as Bcl-2-like protein 4, is a pro-apoptotic protein and prompts cell death by its homodimerization and heterodimerization that is associated with Bcl-2 and other members of the Bcl-2 protein family. Apoptosis is affected by changes in Bcl-2 and Bax expression.

Plants are used as foods such as vegetables, fruits, or spices, which include bionutrients or bioactive phytochemical compounds. From 250,000–500,000 plant species on the earth, only 1–10 % have been studied chemically and pharmacologically for medical approaches, which gave rise to drugs, such as vinblastine, vincristine, taxol, and camptothecin that have an anti-tumor effect and improved the chemotherapy effectiveness in some of the cancers. Among the various medicinal plants, the genus Allium (Family: Amaryllidaceae) has more than 600 species, but only a few species are used as vegetables, food, and spices. Plants of the Allium genus comprise many medicinal species that have been used for the treatment of some diseases. Allium paradoxum (M. Bieb.) G. Don is endemic wild garlic, with limited distribution in Caucasus and Hyrcanian area and native to mountainous regions of northern Iran. It was locally called “Alezi”, known as a wild edible vegetable, traditionally cultured in gardens of people who are living in the northern zone of Iran particularly in Mazandaran province and is used for a preparation of some local foods. (Fig. 1). Recently, researchers have shown some properties of this plant such as cysteine sulfoxides, alliinase activity, antihemolytic, and antioxidant effect and high content of phytochemicals and minerals with health benefits. This herb in traditional medicine of Iran is used as a regulator of cholesterol and high blood fat, reinforcing the body, digestive system, and circulatory system. This plant is suggested to have a possible cytotoxic activity. To the best of our knowledge, there is no scientific report about anticancer activities of this species. In the present study, we have investigated the in vitro pro-apoptotic and cytotoxicity activities of the hydroalcoholic extract of A. paradoxum (M. Bieb.) G. Don against MCF-7 cell lines.

Materials and methods

Collection, processing, and extraction

For drug treatment, plant leaves of A. paradoxums (M. Bieb.) G. Don were collected from the Mazandaran Province, the northern area of Iran (Fig. 1). After harvesting, plants were washed with...
fresh water and then air-dried at room temperature and extracted with the solvent ethanol: water 80:20 v/v and plant: solvent ratio 1:10 for four days using maceration method. After each 24 h, the mixture was filtered and the fresh solvent was added to plant residue. The prepared extract was concentrated to dryness by vacuum distillation apparatus below 40 °C. The pure extract of A. paradoxum was kept in dark at 4 °C until used for cell treatment.

**Cell cultures**

The current study was conducted using breast cancer cell line (MCF-7). The cell line was obtained from the National Cell Bank Pasteur Institute (Tehran, Iran). The cells were cultured in RPMI 1640 medium containing, 2 mM L-glutamine, 10 % FBS, 10 unit/mL penicillin, 100 μg/mL streptomycin; then incubated at 37 °C and 5 % CO\textsubscript{2}.

**Determination of cell viability by MTT assay**

In this study, to determine optimum effects of the extract on cells two variables were considered: concentration and time. The cancer cells were treated with the extract at concentrations of 50, 100 and 200 μM and then respectively were examined after 12, 24 and 48 h. The mentioned concentrations were prepared from 2000 μg/mg stock of solvent extract in PBS buffer, and the experiments were performed in triplicates. Untreated MCF-7 cells were considered as control. Cell survival after incubation time was evaluated by assessing formazan formed by MTT reduction. The tetrazolium-based colorimetric assay (MTT) was performed in the cancer cell line to determine cell viability. After two cell passages, the cells were transferred to 96-well plate (5000 cells per well). When the yellow MTT enters the living cells, it is reduced by dehydrogenase of these cells and produces a purple precipitate. Briefly, after glucose treatment of cells, MTT solution (5 mg/mL in PBS) was added to the wells of 96-well plate and the cells were incubated at 37 °C for 3 h. After the incubation time, the plate containing the cells was centrifuged at 1000 g for 10 min. Then supernatant of the wells was aspirated and 100 μL of DMSO solution was added to each well and in order to dissolve purple MTT precipitates, the plate was agitated for 10 min. Then ODs of the samples were measured at 570 nm by ELISA reader. To calculate the cytotoxic effect of the extract on the survival of MCF-7 cells and to evaluate the death rate of treated cells, the following formula was used:

\[
\text{Cell death rate (\%)} = 1 - \frac{\text{OD}_{\text{exp}}}{\text{OD}_{\text{cont}}} \times 100
\]

In this formula, \(\text{OD}_{\text{exp}}\) and \(\text{OD}_{\text{cont}}\) indicate optical absorption of treated cells and untreated cells (control), respectively.

The MCF-7 cells were incubated with 200 μM concentration of A. paradoxum (M. Bieb.) G. Don extracts for 24 h and then control and treated cells were centrifuged and rinsed with Phosphate Buffered Saline (PBS). Afterward, cell lysis solution (containing 1 % NP-40, /5 % SDS, 10 mMTris-HCl, \(pH = 7.4\)), 150 mMNaCl, 5 mM EDTA, 5% Sodium deoxycholate, 100 mM PMSF and protease inhibitor, were added into plate of each cell group (containing \(5 \times 10^6\) cells) and the samples were placed on ice for 30 min. Each 5 min the samples were vortexed to be homogenized well. Subsequently, the samples were centrifuged (at 13000 rpm and 4 °C) in order for the cell lysis residues to precipitate. The supernatant was removed and the protein concentration was measured using the Bradford method. Protein samples were split into smaller equal volumes and were kept at -80 °C until time of the experiment.

**Assessment cell morphology changes**

For investigation the effects of A. paradoxum (M. Bieb.) G. Don hydroalcoholic extracts on cell morphology, MCF7 cells were seeded in 6 well plates (3 \(\times\) 10\textsuperscript{5}). After 24 h, the cells were treated with different concentrations of plant extracts for 24 h. At the end of the treatment period, the morphology of the cells was determined by fluorescence microscope.

**Cell death**

To determining the type of cell death, the \(2 \times 10^5\) treated cells were double stained with a mixed dye containing acridine orange (AO) and ethidium bromide (EtBr) which were used as fluorescent probes to differentiate between apoptotic and necrotic cells according to the method described by Pornour et al.\textsuperscript{28}. In live cells, both normal and apoptotic nuclei had bright green fluorescence, but in contrast, in dead cells, bright orange fluorescence was seen. Also, ethidium bromide was only taken up by nonviable cells and made to appear dark orange. The living cells have a compressed chromosomes, intact nuclei membrane, and cell membrane. Apoptotic, necrotic, and living cells have different morphology and color. The normal cells appeared uniformly green, and apoptotic cells incorporated by ethidium bromide and therefore stained orange with condensed and often fragmented nuclei.
Evaluation of gene expression by real time RT-PCR

The cells were incubated in cell culture medium for 18 h with 200 µM concentration of *A. paradoxum* (M. Bieb.) G. Don extracts were collected and cellular RNA was extracted after 12, 24 and 48 h, and their quantity was measured by spectrophotometry using the Nano Drop ND-1000 spectrophotometer. For RNA extraction from the studied cells, Tripure Isolation Reagent (Roche) was used according to the instructions. To perform reverse transcription we used Revert Aid First Strand cDNA Synthesis Kit (Fermentas). The required volume to perform this reaction was 20 µL and included: 4 µL PCR 5X buffer, 2 µL dNTP, 1 µL Random Hexamers, 1 µL DEPC treated water, 1µL RNase inhibitor (20 U/µL) and 1µg RNA for each reaction. The above contents were incubated for 5 min at 65 °C, 5 min at 25 °C and one hour at 42 °C and finally, after 5 min of incubation at 70 °C, cDNA synthesis reaction terminated. The synthesized cDNA was stored at -20°C.

The real-time PCR reaction was performed in Rotor Gene 6000 Real Time PCR system and at the volume of 20 µL. For each reaction were used 10 µL Maxima SYBR Green Master Mix (Fermentas), 2 µL cDNA product, 0/5 µL of each primer (20 nmol) and 7 µL Nuclease-free water. The used condition involves one step of primary activation for 30 sec at 95 °C and then 45 cycles of denaturation (5 sec at 95 °C) and combined annealing/extension step (20 sec at 60 °C). Melt-curve analysis was used to determine the specificity of the amplified product. Finally, we used \([2^{-\Delta\Delta CT}}\) formula for comparative quantification of mRNA copy numbers. The sequences of used primers are given in Table 1.

Protein extraction, SDS-PAGE and blotting techniques

After, the collection of protein samples, and mixed with 6X sample buffer containing 62 mM Tris-HCl (pH = 6.8), 2 % SDS, 25 % glycerol, 5 % mercaptetoethanol and 2 % bromophenol blue and boiled for 5 min at 95 °C, the same concentration of extracted proteins from control and treated samples were run on SDS-PAGE electrophoresis. The protein bands were transferred onto nitrocellulose membrane by blotting method. SDS-PAGE and blotting techniques were performed by Mini-Protean 4 Cell (vertical protein electrophoresis) and Mini-Trans-Blot (Bio-Rad) apparatus. Protein band positions and efficiency of transfer were examined by Ponceaues staining and band of interest position was controlled using molecular weight marker. For identifying bands of the proteins Bax and Bcl-2, primary antibodies (USA, Santa Cruz Biotecnology) against them were used. The primary antibody was recognized by peroxidase (HRP) labeled antibody and protein band of interest appeared on film by chemiluminescence (ECLTM Advance Western Blotting Detection Kit, Amersham). Primary antibody against β-actin was used as an internal control.

Statistical analysis

All experiments were conducted in triplicate. For statistical analysis, we used SPSS 16 software package for Windows. Statistical differences between control and experiment groups were examined using Paired Students t-test. Values of p < 0.05 were considered to be statistically significant.

Results

Cell survival evaluation by MTT assay indicated that *A. paradoxum* (M. Bieb.) G. Don hydroalcoholic extracts reduce proliferation of MCF-7 cells in a dose and time-dependent manner (Fig. 2). As it is illustrated in Fig. 2, after 24 h treatment in the presence of different concentration of *A. paradoxum* (M. Bieb.) G. Don hydroalcoholic extract, the concentration of 50 µM of the extract doesn’t have much effect on cell survival whereas the concentration of 100 µM caused significant inhibition of MCF7 cell viability.

<table>
<thead>
<tr>
<th>Table 1—Real-time PCR primers</th>
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<tbody>
<tr>
<td><strong>Genes</strong></td>
</tr>
<tr>
<td>Bcl-2(F)</td>
</tr>
<tr>
<td>Bcl-2(R)</td>
</tr>
<tr>
<td>Bax(F)</td>
</tr>
<tr>
<td>Bax(R)</td>
</tr>
<tr>
<td>B-actin(F)</td>
</tr>
<tr>
<td>B-actin(R)</td>
</tr>
</tbody>
</table>

Fig. 2—Dose and time-dependent manners of MCF-7 cell line by *A. paradoxum* (M. Bieb.) G. Don
The morphological change of MCF7 cells was detected using fluorescence microscope after 24 h treatment. The results showed cells treated with *A. paradoxum* (M. Bieb.) G. Don hydroalcoholic extracts had significant morphological changes in comparison with controls. These changes include: cell contraction and reduction in size, volume, and density of the cells. In AO/ EtBr double staining, the green color indicated living cells without treatment, while yellow and red indicated cells that are at the early and late of apoptotic cells. Fig. 3a shows cells are stained green in the untreated group, while in the apoptotic cells yellow, orange and red staining were observed (Fig. 3b) which suggest that *A. paradoxum* (M. Bieb.) G. Don hydroalcoholic extracts significantly induced the apoptosis in MCF7 cells.

The Bax protein that acts as a key protein in apoptosis is induced by different factors in the intrinsic pathway of apoptosis. To examine the effect of *A. paradoxum* (M. Bieb.) G. Don hydroalcoholic extracts, on the expression of Bax and Bcl-2 genes involved in cell death of MCF7 cells, mRNA expression of the genes was assessed by real-time PCR (Table 1). *A. paradoxum* (M. Bieb.) G. Don extracts up-regulate expression of the Bax gene in a statistically significant manner, whereas has no effect on Bcl-2 gene expression as compared to control group (Table 2).

To confirm real-time PCR results, *A. paradoxum*-induced increase in Bax protein expression in MCF7 cells was assessed using western blot analysis of Bax and Bcl-2 expression. The results indicated that expression of Bax and Bcl-2 genes occurred in accordance with mRNA expression result and there was a statistically significant increase in Bax protein expression, while there was no change in Bcl-2 protein level (Fig. 4).

**Table 2—Real time result of BAX and BCL-2 in different dose and time**

<table>
<thead>
<tr>
<th>Gene</th>
<th>p-value</th>
<th>Rate of changes</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bax</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bax-12h-50</td>
<td>0.046*</td>
<td>4.63</td>
<td>±1.10</td>
</tr>
<tr>
<td>Bax-24h-50</td>
<td>0.001***</td>
<td>5.16</td>
<td>±1.12</td>
</tr>
<tr>
<td>Bax-48h-50</td>
<td>0.001***</td>
<td>5.01</td>
<td>±1.10</td>
</tr>
<tr>
<td>Bax-12h-100</td>
<td>0.070ns</td>
<td>6.86</td>
<td>±1.64</td>
</tr>
<tr>
<td>Bax-24h-100</td>
<td>0.001***</td>
<td>8.98</td>
<td>±2.01</td>
</tr>
<tr>
<td>Bax-48h-100</td>
<td>0.001***</td>
<td>10.50</td>
<td>±2.40</td>
</tr>
<tr>
<td>Bax-12h-200</td>
<td>0.070ns</td>
<td>5.21</td>
<td>±1.29</td>
</tr>
<tr>
<td>Bax-24h-200</td>
<td>0.001***</td>
<td>9.36</td>
<td>±2.05</td>
</tr>
<tr>
<td>Bax-48h-200</td>
<td>0.001***</td>
<td>9.11</td>
<td>±2.14</td>
</tr>
<tr>
<td>Bcl-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcl2-12h-50</td>
<td>0.191ns</td>
<td>-0.48</td>
<td>±0.07</td>
</tr>
<tr>
<td>Bcl2-24h-50</td>
<td>0.625ns</td>
<td>-0.19</td>
<td>±0.09</td>
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<tr>
<td>Bcl2-48h-50</td>
<td>0.804ns</td>
<td>-0.08</td>
<td>±0.21</td>
</tr>
<tr>
<td>Bcl2-12h-100</td>
<td>0.001***</td>
<td>-2.5</td>
<td>±0.59</td>
</tr>
<tr>
<td>Bcl2-24h-100</td>
<td>0.045*</td>
<td>-4.43</td>
<td>±0.75</td>
</tr>
<tr>
<td>Bcl2-48h-100</td>
<td>0.001***</td>
<td>-4.82</td>
<td>±1.10</td>
</tr>
<tr>
<td>Bcl2-12h-200</td>
<td>0.016**</td>
<td>-4.31</td>
<td>±0.87</td>
</tr>
<tr>
<td>Bcl2-24h-200</td>
<td>0.045*</td>
<td>-2.9</td>
<td>±0.72</td>
</tr>
<tr>
<td>Bcl2-48h-200</td>
<td>0.001***</td>
<td>-3.31</td>
<td>±0.70</td>
</tr>
</tbody>
</table>

Notes: Ns: no significant, *: Significant at p ≤ 0.05 level, **: Significant at p ≤ 0.001 level, ***: Significant at p ≤ 0.001 level.

**Discussion**

Breast cancer is the most common malignancy in women worldwide and there is an increasing incidence of this malignancy in the majority of the countries in the world including Iran. Among the causes related to the increase in affliction to cancer, environmental factors, such as air pollution, stress,
lifestyle and diet can be mentioned. It has been elucidated that consumption of foods with bioactive properties has an effective role in cancer prevention and decreasing cancer incidence\textsuperscript{30-31}. So according to the mentioned cases, there has been an increasing trend toward consumption of natural products and nutritional supplements with anti-cancer properties, in recent years. Among the most important of medicinal plants thyme, onion and nettle are intensively used in traditional medicine in the middle and South-West Asia. Garlic is the most wonderful medicinal plant which is extensively used in the daily diet especially in Iran, Asia and has reported preventive characteristics in cardiovascular diseases, regulating blood pressure, lowering blood sugar and cholesterol levels, effective against bacterial, viral, fungal and parasitic infections, against headache, cold, stomach problems, enhancing the immune system and having antitumoral and antioxidant features\textsuperscript{32-38}. Also, recent medicine and pharmaceutical studies showed that the extract of \textit{Allium paradoxum} (M. Bieb.) G. Don can be mentioned among noteworthy nutritional supplements. These findings agreed with the report of Dehghan \textit{et al.}\textsuperscript{39} that exhibited the antioxidant and antidiabetic activities of n-hexane, ethyl acetate, and methanol extracts of various parts of 11 herbal plants from Hrycania region, Iran, including \textit{Allium paradoxum}. In addition, Nabavi \textit{et al.}\textsuperscript{40} showed the hepatoprotective activity of \textit{Allium paradoxum} (M. Bieb.) G. Don and Nabavi \textit{et al.}\textsuperscript{21} reported the recovery effects of \textit{Allium paradoxum} (M. Bieb.) G. Don on gentamicin-induced nephrotoxicity in mice. In the present study, the results in line with previous studies, confirm anticancer activity of this herbal product. This study, suggests that treatment of MCF-7 cells with \textit{Allium paradoxum} (M. Bieb.) G. Don hydroalcoholic extracts reduce proliferation of these cells in the dose and time-dependent manner. The result indicated that this fraction showed a high cytotoxic effect in studied cancer cell lines and reinforcing the notion of the presence of compounds with profound anticancer potential. In accordance with result of current study, Nema \textit{et al.}\textsuperscript{41} considered that the anticancer activity of garlic \textit{Allium sativum} (Bulb) polyphenolic compound on MCF-7, A549 and PA-1 cancer cell line (breast, lung and ovary cancer, respectively), showed efficient cytotoxicity on MCF-7 (6 ± 1 µg ) than PA-1 (15 ± 1 µg) and A459 (28 ± 1 µg) cancer cell line. Mature garlic extracts display the scavenging activities by two most important compounds as S-allylmercapto-Lcysteine and S-allylcystine\textsuperscript{42}. The organic (ethanol) extracts of dry and fresh shallot bulb of \textit{Allium ascalonicum} has been also reported by Pandurangan \textit{et al.}\textsuperscript{43} to exhibit anticancer efficacy. Based on the Ayaz \& Alpsoy\textsuperscript{32}, report from nutritional view-point, garlic extracts have an impressive profile of high-value nutrients and more than 200 chemical compounds such as sulfur (allicin, alliin and agoene), volatile oils, enzymes (allinase, peroxidase and miracynase), carbohydrates (sucrose and glucose), minerals (selenium), amino acids such as cysteine, glutamine, isoleucine and methionine which help to protect cells from the harms of free radicals, bioflavonoids such as quercetin and cyanidin, allistatin I and allistatin II, and vitamins C, E and A which help to protect us from oxidation agents and free radicals, and other vitamins such as niacin, B1 and B2 and betacarotene\textsuperscript{32}.

It has been elucidated that apoptosis is controlled by many extracellular and intracellular factors, which among intracellular factors, the balance between Bcl-2 (anti-apoptotic) and Bax (pro-apoptotic equivalent of Bcl-2) has been introduced as the most important parameter determining cells destiny in response to extracellular stimulus\textsuperscript{44}. In the studies of the cytotoxic and apoptotic effect of natural compounds in cancer, apoptosis is an important mechanism\textsuperscript{45}. The protein, Bax acts as a key protein in apoptosis induced by various factors in the intrinsic pathway of apoptosis. This protein through interaction with mitochondrial membrane proteins leads to increased mitochondrial membrane permeability and release of cytochrome C from mitochondria and caspase activation and eventually apoptosis. On the other hand, Bcl-2 exerts as an anti-apoptotic effect in response to different stimuli through cytochrome C release prevention. Bcl-2 is one of the best cell death control proteins; its overexpression confers resistance to a wide range of apoptosis inducers and the cell survival functions of Bcl-2 are activated by translocation in lymphomas and overexpression in many other cancer types\textsuperscript{10}. The Bcl-2 family of proteins that consists of antiapoptotic and pro-apoptotic members determines life-or-death of a cell by controlling the release of mitochondrial apoptogenic factors, cytochrome c and apoptosis-inducing factor (AIF)\textsuperscript{46}. Bcl-2 exerts an anti-apoptotic function in response to various apoptosis stimuli through prevention of cytochrome C release from mitochondria\textsuperscript{47}. Apoptosis assay by Bax and Bcl-2 genes expression was performed to examine the
effects of A. paradoxum (M. Bieb.) G. Don extracts on apoptotic rates of MCF-7 cells after treatment. The results suggest that A. paradoxum (M. Bieb.) G. Don extracts upregulate expression of the Bax gene mRNA whereas, it has no effect on Bcl-2 gene expression. Also as expected, there was a significant increase in expression of the Bax protein in accordance with the mRNA expression result, while there was no change in Bcl-2 level. Previous literature review revealed that, this functionality of Allium plants extracts might be ascribed to the presence of naturally occurring products, organosulfur or sulfur-containing compounds (OSCs), especially garlic compounds (GCs) and isothiocyanates (ITCs), represent two important and promising chemopreventive in various in vivo and in vitro models, and allicin-decomposition products (diallyl disulfide, diallyltrisulfide, and ajoene), S-allyl cysteine was shown to have potential anticancer activities and may be responsible for some beneficial properties of these plants.

Conclusion
This study was performed to examine the possible potential of pro-apoptotic and cytotoxicity effect of A. paradoxum (M. Bieb.) G. Don extracts against MCF-7 cell line. The finding, emphasize that A. paradoxum, which contains many beneficial phytochemicals, significantly affects the viability of cancer cells. However, further research is essential for discovering the molecular mechanisms of action of active compounds from Allium plants.

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