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# Anti-proliferative activity of *Artemisia marschalliana* on cancerous cell lines

Haleh Forouhandeh[1], Vahideh Tarhriz[1], Masoumeh Zadehkamand[2] and Parina Asgharian[3,4*]

## Abstract

**Background** The genus *Artemisia* of the *Asteraceae* family has different species that are used in the treatment of a wide range of diseases, including cancers due to the presence of valuable compounds and important medicinal properties. Various studies on the anti-tumor effect of different species of *Artemisia* have proven the cytotoxic properties of these plants in cancer treatment, and several anti-cancer compounds of this genus have been purified.

**Objective** The objective of this study was to investigate the cytotoxicity and related mortality mechanisms of *Artemisia marschalliana* essential oil and extracts.

**Methods** The essential oil and various extracts of *Artemisia marschalliana* were elicited using a Soxhlet extractor. Anti-cancer to anti-proliferative activity as MTT assay is measuring cancerous and non-cancerous cell viability. In the next step, the strongest extract fractions were obtained by using the vacuum liquid chromatography method. Flow cytometry was applied to identify the mechanism of cell death, and a Real-time polymerase chain reaction test of apoptosis genes, which encode apoptosis-regulating proteins, was measured to confirm the flow cytometry results.

**Results** The strongest extract belonged to dichloromethane extract 60% fraction of the extract on breast cancer cells and 80% fraction on liposarcoma cancer cells showed the most cytotoxicity within 48 h, while, the fractions did not notable cytotoxicity of non-cancerous cells cell. Flow cytometry analysis illustrated the mentioned extract and its fractions kill cancer cell lines through the apoptosis mechanism. Our findings confirmed the flow cytometry results. In addition, the essential oil of *Artemisia marschalliana* showed a considerable cytotoxic property.

**Conclusion** Dichloromethane extract of *Artemisia marschalliana* shoot and its 60 and 80% fraction selectively inhibited the growth of cancer cells by inducing the apoptosis mechanism. Regarding obtained results, 60 and 80% fractions of dichloromethane extract can be a good candidate for future studies in the field of identification and separation of pure cytotoxic compounds.

**Keywords** *Artemisia marschalliana*, Cytotoxic activity, Apoptosis, Cancerous cells

*Correspondence:
Parina Asgharian
parina.asgharian@gmail.com
[1] Molecular Medicine Research Center, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Student Research Committee, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[4] Drug applied research center,, Tabriz University of Medical Sciences, Tabriz, Iran

## Introduction

Cancer is the abnormal growth of cells that can invade and metastasize to other parts of the body [1, 2]. The disease is still one of the leading causes of death worldwide [3]. In 2022, 1,918,030 new cases of cancer and 609,360 cancer deaths are projected in the United States [4]. It is estimated that one-third of cancer death is due to five important risk factors including high BMI (Body mass index), not consuming fruits and vegetables, low physical activity, tobacco, and alcohol consumption [5].

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Liposarcoma (LS) is a common type of soft tissue sarcoma [6, 7]. In addition, breast cancer is one of the most important and common malignancies in Iranian women [8]. The type of cancer and the rate of its progression will determine the treatment methods. Surgery, chemotherapy, radiotherapy, immunotherapy, gene therapy, cell therapy, and hormone therapy are common methods to treat cancer. However, these treatments face limitations [9]. Medicinal plants have long been the most important source of treating various diseases. Today, cytotoxic secondary metabolites and their semi-synthetic derivatives have a special place in cancer therapies, therefore, the study of medicinal plants has been considered by researchers around the world [10, 11]. Among the known medicinal plants, the genus *Artemisia* of the family *Asteraceae* has noteworthy compounds for their following effects: antioxidant activity [12], anti-inflammatory properties, antimalarial, antiviral, antibacterial, antifungal, and antitumor effects [13]. It should be noted that the anti-cancer effect of artemisinin and its derivatives has been proven in various studies [14, 15]. Significant antitumor compounds in *Artemisia* include monoterpenes, terpenes, and phenolic compounds [13]. Apoptosis is the predominant effect of extracts or active ingredients of Artemisia species. These species induce apoptosis in different cell lines by activating caspases, depolarizing the mitochondrial membrane potential, and reducing BCL-2 expression [16] or stopping the cell cycle [17, 18]. Hu et al. observed the usual morphological changes of apoptosis, including dense chromatin and volume reduction by exposure to another human hepatoma cell line with components of *Artemisia capillaries* Thunberg [17]. Based on previous reports, *Artemisia* species have various biologically active compounds and different medicinal properties of their unique compounds in this genus. To achieve this goal and considering the presence of valuable compounds in the genus *Artemisia*, the present study investigated to explore the cytotoxic effects of different extracts of *A. marschalliana* on cancer cells (MCF-7 and SW872) and normal cells (MCF-10A).

## Materials and methods
### Preparation of plant samples
Plant *A. marschalliana* (synonym of *Artemisia campestris subspindora*) was collected in September 2016 from the Kalibar region of East Azerbaijan province, Iran, (38°52′04.5″N 47°02′26.4″E). The plant was identified by Dr. Fatemeh Ebrahimi at the herbarium of Tabriz University of Medical Sciences, Tabriz, Iran (Code number: TBZ-FPH 4037). *A. marschalliana* is freely available and used by local people as a plant in traditional medicine and there is no constraint by the authority to collect the plant. The experimental research and field studies on plants, including the collection of plant material, comply with relevant institutional, national, and international guidelines and legislation, which were approved by the Research Ethical Committee (REC) Numbers: IR.TBZMED.RDC.1396.1187 and IR.TBZMED.RDC.1397.012, Tabriz University of Medical Sciences, Tabriz, Iran.

### Preparation of extracts
The shoots of *A. marschalliana* were washed carefully and dried in the open sterile air condition. The dried plants were powdered using an electric mill for subsequent experiments. After the shoots of *A. marschalliana* were dried in a laboratory under an electric mill, 200 g of shoot powder were extracted by Soxhlet apparatus with solvents of n-Hexane (n-Hex), dichloromethane (DCM), and methanol (MeOH). The obtained extracts were dried to remove solvents completely by using a rotary evaporator at 45 °C [19].

### Preparation of DCM extract fraction by VLC method
We filled the VLC hopper with silica gel using a vacuum pump and after washing with 150 mL of methanol and ethyl acetate solvents, loaded 2 g of the plant sample into it. Then, washed the funnel with 200 mL of a mixture of ethyl acetate and hexane solvents with increasing polarity (10, 20, 40, 60, 80, and 100%), and the output of each wash in a separate container. Finally, we poured and rotated them with an evaporator at 45 °C [19].

### Essential oils
First, we poured 150 g of dried and ground plant powder into the jouette balloon, and then poured up to two-thirds of the balloon volume in the ratio of 1 to 3 glycerin and water on the plant powder. To prevent heat loss, we covered the Clevenger tube with heat insulation. The total time of essential oil collection was 4 h and the maximum amount of essential collected oil was in the first hour [20].

### Cell lines used in experiments
Human breast cancer cell lines with estrogen, progesterone, and glucocorticoid receptors (MCF-7), undifferentiated malignant tumors consistent with human liposarcoma cells (SW872) were used as cancerous cell lines and normal human mammary epithelial cells (MCF-10A) was used as the control cells. The cell lines were purchased from the National Cell Bank, Pasteur Institute of Iran.

### Anti-proliferative activity of extracts by MTT assay
The substance MTT (dimethylthiazole-2 and 5-diphenyltetrazolium bromide) turns into insoluble purple

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formazan crystals due to the cleavage of the tetrazolium ring by mitochondrial enzymes in living cells. Therefore, to determine extracts toxicity on cell lines, and calculate of $IC_{50}$ value, MTT assay was used [21]. RPMI 1640 culture medium with 10% fetal bovine serum (FBS) (Gibco), and 1% penicillin/streptomycin (Gibco), was used for cell culture experiments. To perform this test, 15,000 cells we spread into each well of a 96-plate with a volume of 200 μL. After 24 hours, the cells adhered to the bottom of the plate. The different concentrations of extract including 10, 20, 50, 100, 150, 200, and 300 μg/mL were treated on the well in three series of repetitions. Taxol was considered a reference drug control and Dimethyl sulfoxide (DMSO) as a positive control. The cells were incubated at 37 °C, 5% CO2, and 95% humidity for 24 and 48 hours, and then the culture medium is drained by a sampler, and 50 μL of MTT solution with a concentration of 2 mg/mL and 100 μL of complete culture medium was added to each well. This should be done in very low light, then we wrap the plate in aluminum foil and put it in the incubator for 4 hours. After 4 hours of incubation, the content of the 96-well plate was emptied, and by adding 200 μl of DMSO, the formazan crystals were dissolved and the purple color created by the plate reader was measured at a wavelength of 570 nm [22].

### Detection by flow cytometry

The number of dead cells treated with different extracts via the apoptosis mechanism was determined by flow cytometric assay kit (BD bioscience, NJ, USA) at 488 (excitation wavelength) and 617 nm (emission wavelength) [23, 24]. To perform a flow cytometry test, we added $2 \times 10^5$ cells to each 6-cell well plate. Then, added 150 μL of trypsin and dilute with 50 μL of PBS. One hundred microlitre of the Annexin V binding buffer with deionized water (1: 10) with along 5 μL of Annexin V and 5 μL of Propidium Iodide were added to each microtube except for unstained control. Non-color control was used to remove any background adsorption and color control was used to prevent natural cell apoptosis or necrosis from interfering with the results of the samples [24].

### Quantitative RT-PCR analysis and western blotting tests

Total RNA was extracted using RNX-Plus reagent (Cinnagen, Co. Tehran, Iran) from treated and non-treated cells according to the manufacturer protocol, and single-strand cDNAs were synthesized using a random hexamer and oligo dT primers using SinaClon, Cat. No.: RT5201. RT-PCR was carried out for *P53* gene in the presence of specific primers (forward: 5′-CCCATCCTCACCATC ATCACAC-3′ and reverse: 5′-GCACAAACACG CAC CTCAAAG-3′) and *Bax* gene (forward: 5′-GATGCG TCCACCAAGAAGCT-3′ and reverse: 5′-CGGCCC

CAGTTGAAGTTG-3′) [22, 25] using TaKaRa master mix (Cat, No: RR820L) [26]. GAPDH mRNA level was used to normalize the genes under study in the presence of primers (forward, 5′-ATGACTCTACCCACG GCAAG-3′); (reverse, 5′-CTGGAGATGGTGATGGGT T-3′) and the relative quantity of mRNA samples was detected using the standard ΔΔCt method [26, 27]. To confirm RT-PCR results, BAX and BCL-2 protein levels were evaluated in treated and non-treated cells. For this aim, whole cell contents were isolated using a lysis buffer containing 500 μl of Tris-Hcl: 500 μL, EDTA: 3 mg, NaCl: 80 mg, Deoxycholic acid sodium salt (DOS): 25 mg, SDS: 10 mg, and 10 μL of 1% Triton with protease inhibitory cocktail [28, 29]. The concentration of isolated proteins was determined using the Bradford concentration method. Then 12 μl of protein was separated on 5% SDS-PAGE gel to isolate proteins and replace nitrocellulose membrane (PVDF) (Amersham, Cat. No: 10600023) using the semidry electrophoretic transfer method [28, 29]. Blocking solution was then used to prevent non-specific reaction of the initial antibody. Primary antibody in the blocking solution to prevent non-specific reaction added to PVDF and incubated. Furthermore, the secondary antibody was utilized to detect desired protein band [26, 30]. Chemoluminescence kit (ECL™ Advance Blocking Reagent Cytiva, RPN418) was used to visualize the desired protein band [26, 30]. Anti- BAX (B-9): sc-7480, and anti-BCL-2 (N-19): sc-492 in a 1:500 dilution in PBS, 2.5% Blotto, 0.05% Tween-20; β-Actin (C4): sc-47778 1:10,000 dilution in PBS, 2.5% Blotto, 0.05% Tween-20, mouse-IgGκ BP-HRP: sc-516102 1:10,000 in a dilution in PBS, 2.5% Blotto, 0.05% Tween-20 as secondary antibody, and protein ladder- Super Signal® Molecular Weight (MAN0011723) were utilized.

### GC/MS analysis

DCM extract (1 μL) was injected into the apparatus of Shimadzu GCMS-QP5050A equipped with a DB-1 column (60 m × 0.25 mm; film thickness 0.25 μm). The initial temperature (50 °C, 3 min) was raised to 270 °C at a rate of 4 °C/min. The injection temperature was adjusted to 240 °C [31, 32].

### Statistical analysis of data

The half-maximal inhibitory concentration ($IC_{50}$) value of the samples indicated the concentration of the sample, which inhibits 50% of cell growth. *P*-value by ANOVA and Tukey post hoc test analysis in all tests was considered less than 0.05 significant using GraphPad Prism 8.0.1 software.

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## Results

### Amounts of extracts and fractions

The weight of obtained extracts as a percentage was shown in Table 1. In addition, the weight of the strongest extract fractions (DCM extract) was also shown as a percentage in Table 1.

### Investigation of cytotoxic effects

#### *Investigation of cytotoxic effects of the studied plant by MTT assay*

MTT test was performed to determine the anti-proliferative effects of the studied plant. As indicated in Table 2, the low $IC_{50}$ values belonged to DCM extract in both cell lines, which indicated the high anti-proliferative effect of this extract; therefore, DCM extract is fractionated with VLC. Statistical analysis of ANOVA and Tukey post hoc test showed the respective fractions were significantly different from the DMSO control. Data showed that the inhibition of cell growth in both cancer cell lines increases with increasing concentrations of these extracts and fractions, as shown in Fig. 1.

The time-dependent effects of DCM extract and related fractions have been investigated indicated that in the MCF-7 cell line, all plant samples have time-dependent effects, but the effects of cytotoxicity in the SW872 cell line increase significantly only over 40 and 100% fractions over time. The viability of MCF-7 cells in the face of 60% fraction compared to MCF-10A cells is significantly reduced; as a result, it seems that the 60% fraction is the most effective in MCF-7 cell line and had the least adverse effects on normal cells (Fig. 2). 60 and 80% fractions on the SW872 cell line showed the best cytotoxic effect. However, 80% fraction showed a selective cytotoxic effect on the SW872 cell line due to a notable reduction in the viability of SW872 cells compared to MCF-10A cells and was introduced as the best fraction. Furthermore, the essential oil sample of this plant with $IC_{50}$ equivalent to $11.91 \pm 2.59\,\mu g/mL$ inhibited the growth of breast cancer cells. Essential oil yield $(w/w_0)$ was 74.43%.

### *Apoptosis assay results on MCF-7 and SW872 cells*

Flow cytometry tests were utilized to detect the apoptosis rate. The histogram view of apoptosis induction by DCM extract and related fractions in MCF-7 and SW872 cell lines is shown in Figs. 3 and 4. The results revealed that 60% fraction in MCF-7 cell line and DCM extract and 60 and 80% fractions in SW872 cell line had the highest rate apoptosis induction. Moreover, we did not any notable apoptosis or necrosis rate in MCF-10A normal cell line. This valuable finding showed that the effective compounds with anti-cancer features probably belong to the DCM extract and its related fractions.

**Table 1** The weight of n-Hex (n-Hexane), DCM (dichloromethane), and MeOH (methanol) extracts powder and fractions of DCM extract from 200 g of *A. Marshalliana*

| Methanol extract | | Dichloromethane extract | | n-Hexan e extract | | Extract |
|---|---|---|---|---|---|---|
| 10.03 | | 1.25 | | 2.26 | | *Weight of plant extract (g/100 g of extract)* |
| **100%** | **80%** | **60%** | **40%** | **20%** | **10%** | *Fractions* |
| 25 | 10.35 | 3.05 | 2.15 | – | – | *Weight of DCM extract fraction (g/100 g of extract)* |

**Table 2** $IC_{50}$ values of extracts and fractions of the most effective extracts on cancer cells (MCF-7 and SW872) and normal (MCF-10A) after 24 and 48 hours in µg/mL

| | MCF-7 (24 h) | MCF-7 (48 h) | SW872 (24 h) | SW872 (48 h) | MCF-10A (24 h) | MCF-10A (48 h) |
|---|---|---|---|---|---|---|
| n-Hexane extract | $165.4 \pm 8.9$ | $128.1 \pm 10.8$ | $132.3 \pm 3.6$ | $118.7 \pm 8.48$ | $197.7 \pm 13.5$ | $86.34 \pm 0.4$ |
| Dichloromethane extract | $41.74 \pm 9.2$ | $38.61 \pm 0.9$ | $104.4 \pm 2.4$ | $102.2 \pm 2.1$ | $93.83 \pm 0.4$ | $30.7 \pm 0.2$ |
| Methanol extract | $< 800$ | $< 800$ | $< 800$ | $< 800$ | $< 800$ | $< 800$ |
| 40% VLC fraction of Dichloromethane extract | $199 \pm 21.42$ | $39.47 \pm 6.08$ | $259.4 \pm 48.15$ | $231.3 \pm 2.6$ | $142.3 \pm 30.11$ | $133.82 \pm 12.1$ |
| 60% VLC fraction of Dichloromethane extract | $17.86 \pm 2.84$ | $9.42 \pm 1.09$ | $43.72 \pm 4.4$ | $38.52 \pm 4.3$ | $56.67 \pm 1.7$ | $63.75 \pm 3.7$ |
| 80% VLC fraction of Dichloromethane extract | $136.4 \pm 5.3$ | $58.36 \pm 3.91$ | $79.94 \pm 3.9$ | $69.18 \pm 6.7$ | $104.6 \pm 6.8$ | $107.2 \pm 10.6$ |
| 100% VLC fraction of Dichloromethane extract | $156 \pm 0.5$ | $79.39 \pm 4.01$ | $179.5 \pm 6.2$ | $137.4 \pm 6.71$ | $128.9 \pm 0.98$ | $134.1 \pm 6.29$ |
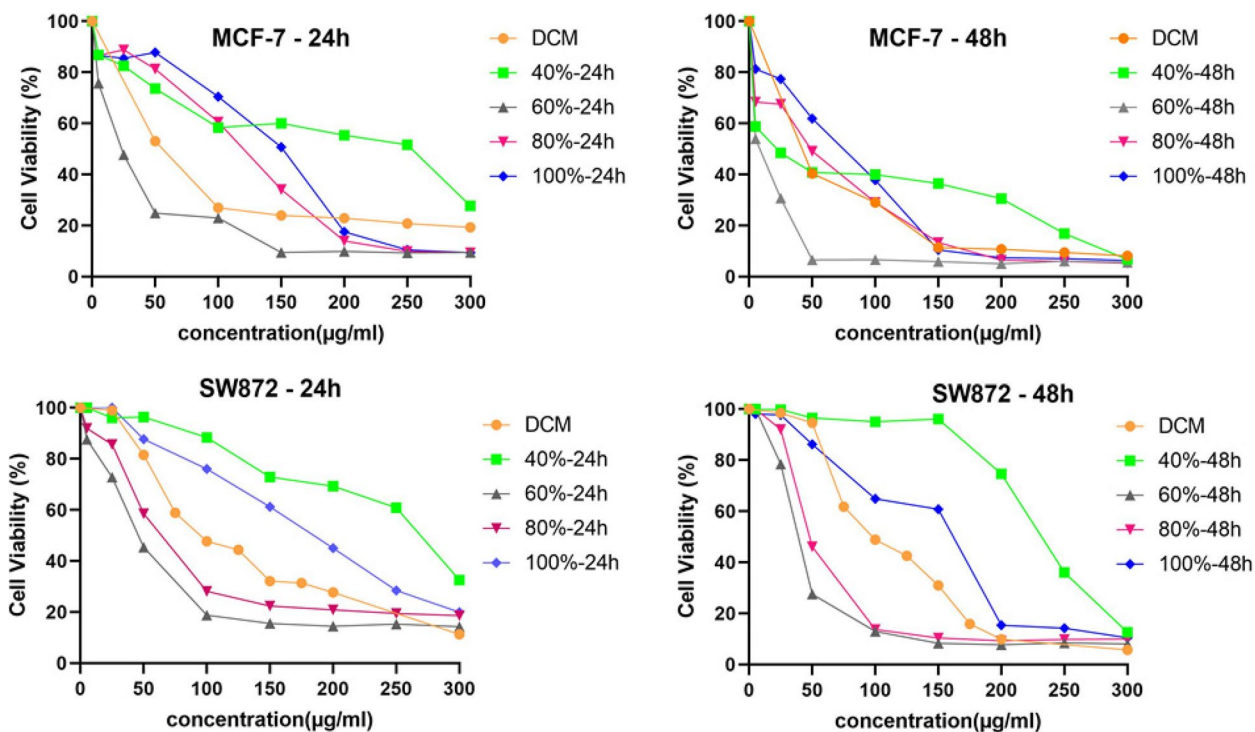
**Fig. 1** The cell viability of MCF-7 and SW872 cancer cell lines in different concentrations of DCM (dichloromethane) extract and its fractions after **a**) 24 and **b**) 48 hours of incubation
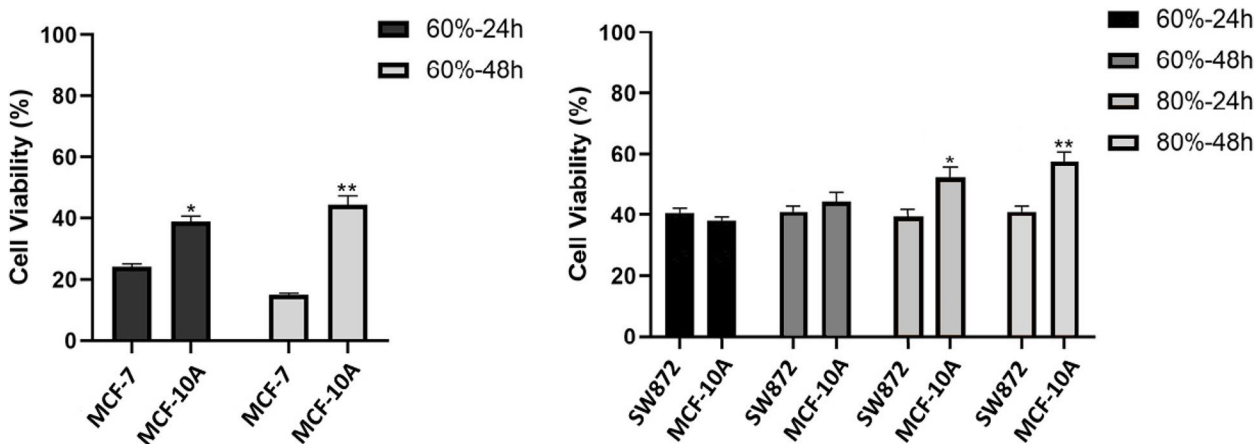


**Fig. 2** The cell viability of MCF-7 and SW872 cancer cells due to exposure to mentioned fractions of DCM (dichloromethane) extract compared to normal MCF-10A cells

### RT-PCR analysis and western blotting results

To confirm the induction of apoptosis by *A. marschalliana* extract in MCF-7 and SW872 cancer cells, the expression level of mRNA of *P53* and *BAX* genes was investigated. After 24 h of exposure to the determined $IC_{50}$ concentration induced by *A. marschalliana* extract, mRNA levels were evaluated by RT-PCR with related primers. The RT-PCR efficiency of all tests was between 1.82–1.93. The data showed the upregulation of *P53* and *BAX* gene expression as the main pro-apoptotic gene. Although the expression levels of *P53* and *BAX* were significantly increased in treated-cancer cells, they had no significant change in non-treated cells (Fig. 5a and b). Furthermore, an increase in BAX protein level based on the results of western blot analysis is strong evidence to proof of previous test results. In addition, there was
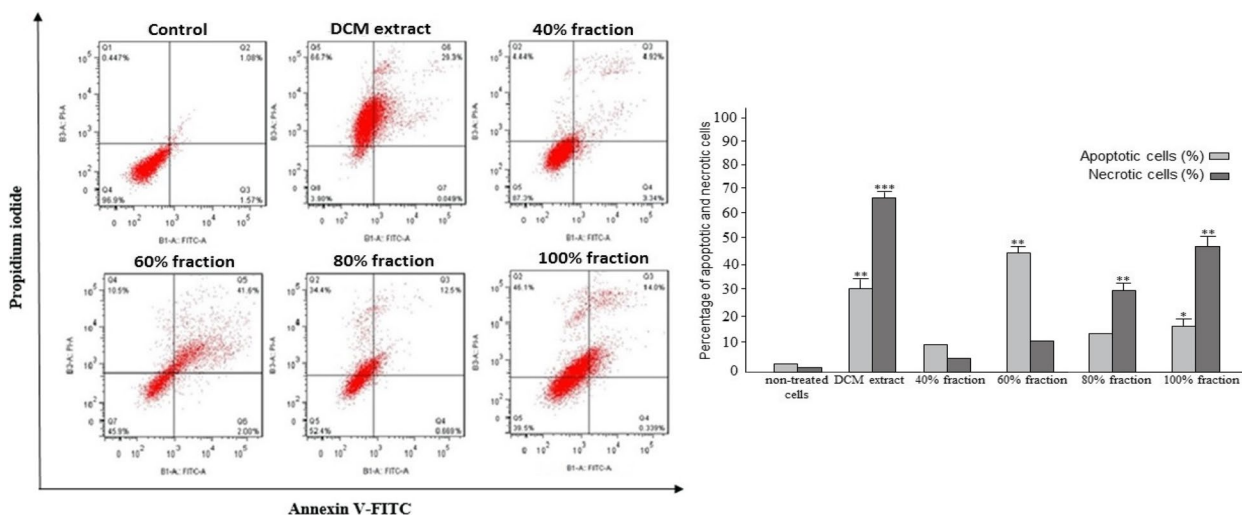
**Fig. 3** The results of Flow cytometric test against MCF-7 cell line treated with DCM (dichloromethane) extract and its related fraction. The graphs were calculated and drawn using statistical analysis and indicated by an asterisk ($p \leq 0.001$ ***, $p \leq 0.01$:**, $p \leq 0.05$: *)



**Fig. 4** The results of apoptosis assay against SW872 cell line treated with DCM (dichloromethane) extract and its related fractions. The graphs were calculated and drawn using statistical analysis and indicated by an asterisk ($p \leq 0.001$ ***, $p \leq 0.01$:**, $p \leq 0.05$: *)
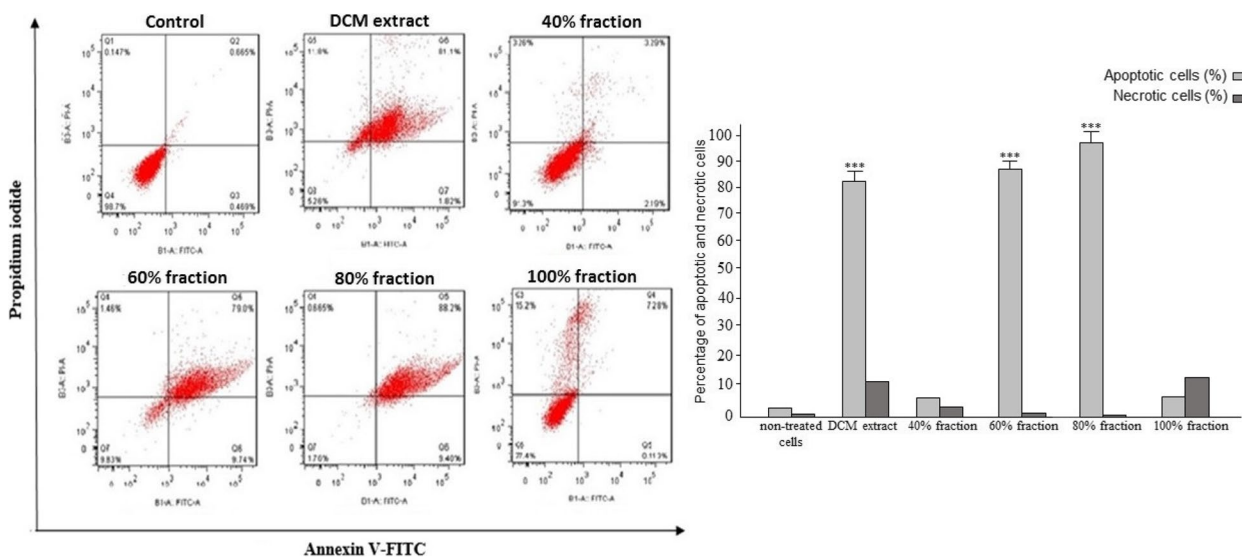
a slight decrease in BCL-2 protein expression level in treated-cancer cells; however, the change was not significant (Fig. 6). These findings indicate the potential efficacy of the extract that is mediated by its ability to induce programmed cell death.

### GC/MS analysis results

GC/MS analysis was performed to obtain the most effective compounds. The results led to obtaining of 45 compounds from the DCM extract. Among the compounds, β-thujone and comphore have the highest amounts

including (152.23 g/mol, Retention time = 14.02 min) and (152.23 g/mol, Retention time = 15.05 min), respectively. It seems that these two compounds as the major compounds play an important role in showing the observed effects (Table 3).

### Discussion

Recently, non-communicable diseases such as cancer are considered the most important cause of death in the world. The disease, which is one of the most common and growing diseases, is a major part of the care system's
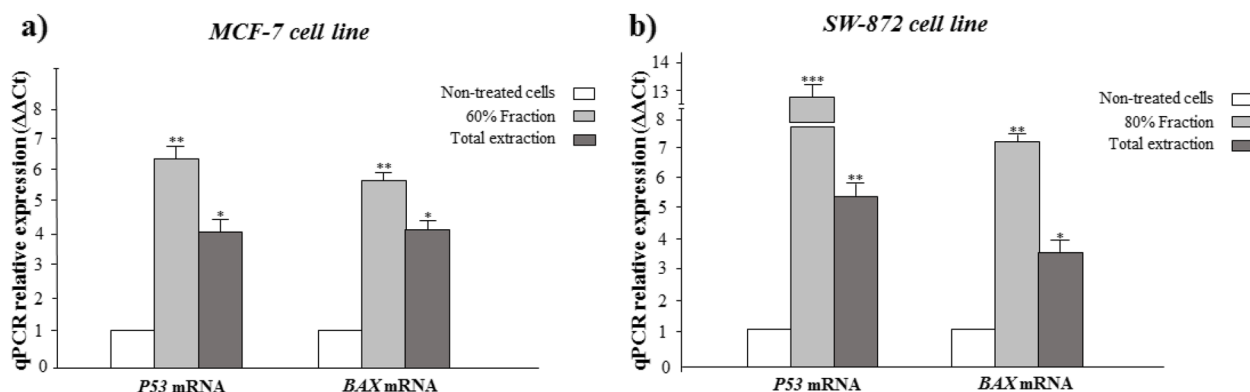
**Fig. 5** The mRNA expression level of *P53* and *BAX* genes in treated MCF-7 and SW872 cancer cells compared to non-treated cells. Data are mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$

efforts, with cancer costing the international community [5, 33]. Liposarcoma is the most common soft tissue sarcoma in adults treated with surgery and radiotherapy; however, the metastatic forms of this cancer only respond to chemotherapy, which is accompanied by inadequate side effects and efficacy, resulting in reduced survival rates [34, 35]. Breast cancer is also the most important cause of cancer death among women due to its high prevalence (1 in 4 cases of cancer in women is related to breast cancer) [5]. Severe side effects are the main difficulty of current chemotherapy drugs. Therefore, there is an urgent need for more studies to find suitable alternatives with fewer side effects [36]. Today, a significant portion of these studies target plants and natural plant-derived products. The use of medicinal plants has been considered due to their biological safety. Many plant compounds have been identified as potential anti-cancer and their number is increasing day by day [37, 38]. On the other hand, most anti-cancer drugs used in chemotherapy regimens are of natural origins, such as the alkaloids vinca, taxanes, and epipodophyllotoxins. Therefore, natural compounds are an important source of research and study in the field of the development of anti-cancer drugs [39]. Plants and their active compounds show anti-cancer effects with antioxidant effects, induction of apoptosis, stopping the cell cycle, inhibiting angiogenesis, and eliminating free radicals. This makes researchers and pharmacists use extracts and active compounds of medicinal plants in the production of new anti-cancer drugs [40]. Due to its anti-bacterial, anti-fungal, and disinfecting properties, the species of Artemisia are widely used in the treatment of some diseases such as malaria, hepatitis, cancer, tumors, neuritis, fever, swelling and wound healing [41]. Various reports have been published about the anti-proliferative activity of fractions and extracts of different species of Artemisia [41–43]. The cytotoxic

effect is caused by secondary metabolites and other non-metallic compounds involved in the synthesis. Apoptosis as an important defense system controlling cancer is regulated by carcinogenic and cancer-inhibitory genes. In the internal pathway of cell death, mitochondria, and the external pathway, death receptors play a major role. Apoptosis in the internal pathway through caspase 9 and in the external pathway through caspase 8 activates caspase 3 and initiates chromosomal degradation. Studies have shown that the *P53* gene plays an important role in apoptosis. Cancer cells use different molecular mechanisms to suppress the apoptosis pathway. For example, increased expression of BCL-2 (anti-apoptotic protein) or decreased expression or mutation in pro-apoptotic proteins such as BAX [44].

Essential oil of *Artemisia annua* can induce apoptosis of cultured SMMC-7721 cells. Artemisinin, a compound of sweet wormwood (*A. annua*), showed selective toxicity to cancer cells in vitro. In addition, it is given orally to delay the growth of breast cancer in mice treated with 7,12-dimethylbenz [α] anthracene (DMBA) [45, 46]. The ability of smoke and aqueous extracts of *A. princeps* to induce apoptosis was examined on MCF-7 human breast cancer cells in vitro. Smoke and water-soluble extracts induce apoptosis through the mitochondrial pathway in breast cancer cells [16]. In this research, we studied the cytotoxicity of *A. marschalliana* against MCF-7 and SW872 cancer cell lines MCF-10A normal cell line. In this regard, after culturing, cytotoxicity and IC$_{50}$ values were obtained from the treatment of these cells with different extracts of n-Hex, DCM, and MeOH. The plant was evaluated in different concentrations and times. DCM extract showed the strongest cytotoxicity on cancer cells compared to n-Hex and MeOH extracts; therefore, fractionation with the VLC method was carried out for this extract and the cytotoxicity of obtained fractions
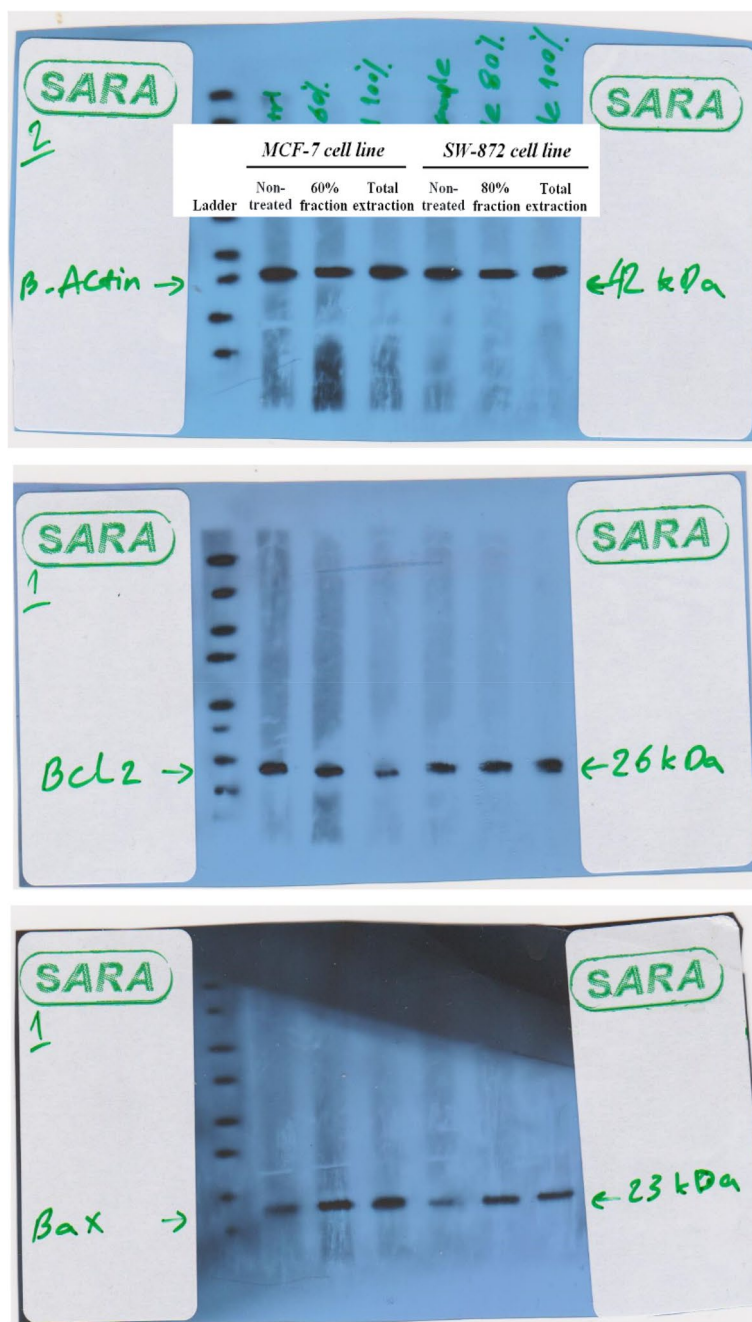
**Fig. 6** The proteomic levels of BCL-2 and BAX proteins in treated MCF-7 and SW872 cancer cells compared to non-treated cells

was evaluated. Because apoptosis or programmed death is one of the natural homeostatic mechanisms of cells in the body and is an important marker in cellular studies for the cytotoxicity of anti-cancer compounds [44], the mechanism of cytotoxicity was evaluated by Flow cytometry annexin V/PI kit. According to our finding, the occurrence of cytotoxic effects depends on the concentration of extracts and fractions ($p < 0.05$) and the increase of the plant samples concentration causes to decrease in the cell viability. In the MCF-7 cell line, the cell viability with treated DCM extract and the mentioned fractions depends on the time of exposure ($p < 0.001$). Regarding time-dependent effects, it can be argued that the active ingredients may be changed from prodrug to active drug over time of incubation and show cytotoxic effects, or after participating in intracellular metabolic pathways,

**Table 3** The GC/MS analysis of the identification of *A. marschalliana* DCM (dichloromethane) extract compounds

| NO | Compounds | Molecular formula | Molecular mass (g/mol) | Retention time (min) | Percent of area | RIa | RIb |
|---|---|---|---|---|---|---|---|
| 1 | Octane | $C_8H_{18}$ | 114 | 3.9 | 0.67 | 802 | 800 |
| 2 | *Cis*-Salvene | $C_9H_{16}$ | 124 | 5.13 | 1.03 | 852 | 852 |
| 3 | *α*-Pinene | $C_{10}H_{16}$ | 136 | 7.47 | 0.7 | 933 | 936 |
| 4 | Tricyclene | $C_{10}H_{16}$ | 136 | 7.8 | 0.74 | 943 | 923 |
| 5 | Camphene | $C_{10}H_{16}$ | 136 | 7.96 | 2.50 | 947 | 949 |
| 6 | Sabinene | $C_{10}H_{16}$ | 136 | 8.83 | 0.8 | 973 | 970 |
| 7 | *β*-Phellandrene | $C_{10}H_{16}$ | 136 | 9.93 | 0.82 | 1005 | 1013 |
| 8 | *α*-Terpinene | $C_{10}H_{16}$ | 136 | 10.36 | 1.07 | 1017 | 1014 |
| 9 | *γ*-Terpinen | $C_{10}H_{16}$ | 136.24 | 11.97 | 1.81 | 1061 | 1062 |
| 10 | *Trans*-Sabinene hydrate | $C_{10}H_{18}O$ | 154.25 | 13.17 | 1.14 | 1094 | 1097 |
| 11 | **β-Thujone** | **$C_{10}H_{16}O$** | **152.23** | **14.02** | **8.80** | **1118** | **1116** |
| 12 | 3-Thujanone | $C_{10}H_{18}O$ | 154.25 | 14.06 | 9.40 | 1119 | 1112 |
| 13 | **Camphor** | **$C_{10}H_{16}O$** | **152.23** | **15.05** | **11.54** | **1146** | **1145** |
| 14 | *(E)-p*-2-Menthen-1-ol | $C_{10}H_{18}O$ | 154.25 | 15.11 | 1.63 | 1148 | 1140 |
| 15 | *Trans*-Bornyl acetate | $C_{10}H_{16}O$ | 152.23 | 17.54 | 2.02 | 1216 | 1174 |
| 16 | 2-Methyl-3-phenyl-propanal | $C_{10}H_{12}O$ | 148.2 | 18.59 | 1.32 | 1247 | 1284 |
| 17 | *D*-Carvone | $C_{10}H_{14}O$ | 150.22 | 18.75 | 1.20 | 1251 | 1234 |
| 18 | Methyl hydrocinnamate | $C_{10}H_{12}O_2$ | 164.2 | 19.77 | 0.74 | 1281 | 1286 |
| 19 | Eugenol | $C_{10}H_{12}O_2$ | 164.2 | 22.62 | 0.76 | 1367 | 1355 |
| 20 | Copaene | $C_{15}H_{24}$ | 204.35 | 22.97 | 1.13 | 1378 | 1382 |
| 21 | Methyl cinnamate | $C_{10}H_{10}O_2$ | 162.18 | 23.63 | 0.70 | 1398 | 1388 |
| 22 | Jasmone | $C_{11}H_{16}O$ | 164.24 | 23.81 | 0.89 | 1404 | 1392 |
| 23 | *γ*-Gurjunene | $C_{15}H_{24}$ | 204.35 | 24.05 | 0.80 | 1412 | 1400 |
| 24 | *p*-Menth-1-en-9-ol | $C_{10}H_{18}O$ | 154.25 | 26.06 | 0.64 | 1476 | 1294 |
| 25 | Germacrene D | $C_{15}H_{24}$ | 204.35 | 26.25 | 4.70 | 1483 | 1480 |
| 26 | γ -Cadinene | $C_{15}H_{24}$ | 204.35 | 26.54 | 0.85 | 1492 | 1493 |
| 27 | Bicyclo germacrene | $C_{15}H_{24}$ | 204.35 | 26.73 | 1.46 | 1498 | 1494 |
| 28 | *β*-Gurjenene | $C_{15}H_{24}$ | 204.35 | 27.02 | 0.74 | 1508 | 1504 |
| 29 | *α* -Amorphene | $C_{15}H_{24}$ | 204.35 | 27.08 | 0.73 | 1510 | 1542 |
| 30 | *σ*-Cadinene | $C_{15}H_{24}$ | 204.35 | 27.55 | 0.88 | 1526 | 1524 |
| 31 | (—)-Spathulenol | $C_{15}H_{24}O$ | 220.35 | 29.22 | 1.49 | 1583 | 1582 |
| 32 | Caryophyllene oxide | $C_{15}H_{24}O$ | 220.35 | 29.34 | 0.94 | 1587 | 1583 |
| 33 | Isoaromadendrene epoxide | C15H24O | 220.35 | 30.17 | 0.76 | 1616 | 1594 |
| 34 | Ledene | $C_{15}H_{24}$ | 204.35 | 31.15 | 0.74 | 1651 | 1656 |
| 35 | Aromadendrene | $C_{15}H_{24}$ | 204.35 | 31.26 | 0.69 | 1655 | 1449 |
| 36 | Vulgarol B | $C_{15}H_{24}O$ | 220.35 | 31.87 | 0.68 | 1677 | 1688 |
| 37 | Isocaryophyllene | $C_{15}H_{24}$ | 204.35 | 32.3 | 0.87 | 1692 | 1404 |
| 38 | Elemol | $C_{15}H_{26}O$ | 222.37 | 33.75 | 0.76 | 1732 | 1542 |
| 39 | Naphthalene | $C_{15}H_{24}$ | 204.35 | 35.15 | 0.66 | 1800 | 1196 |
| 40 | Valerenol | $C_{15}H_{24}O$ | 220.35 | 35.48 | 0.69 | 1813 | 1736 |
| 41 | Hexahydrofarnesyl acetone | $C_{18}H_{36}O$ | 268.5 | 36.36 | 0.77 | 1847 | 1845 |
| 42 | Epimanoyl oxide | $C_{20}H_{34}O$ | 290.5 | 39.95 | 0.76 | 1993 | 2002 |
| 43 | Eicosane | $C_{20}H_{42}$ | 282.5 | 40.09 | 0.68 | 1999 | 2000 |
| 44 | Phytol | $C_{20}H_{40}O$ | 296.5 | 42.79 | 1.07 | 2117 | 2135 |
| 45 | *D*-Nerolidol | C15H26O | 222 | 47.05 | 0.66 | 2314 | 2314 |

RIa: Calculation retention index based on the HP-5MS column

RIb: Retention index in the literature

the active metabolite is more than the main drug. Based on the apoptosis analysis, the rate of apoptosis is the highest value for DCM extract, 60 and 80% fractions in SW872 cell line and 60% fraction in MCF-7 cell. Statistical calculation results illustrated that the viability of MCF-7 cell line, in the face of 60% fraction was significantly different ($p < 0.0001$) from other fractions. It can be concluded that the fraction inhibits 60% of cancer cell growth by inducing apoptosis. The cytotoxic content of this fraction showed the highest rate of apoptosis among the other (43.6%). Moreover, the viability of the 60% fraction decreases over time and the effects of cytotoxicity are time dependent ($P < 0.001$). The viability of the 60% fraction in the incubation times of 24 and 48 hours, in this cell line was significantly different from the MCF-10A cell line ($p < 0.05$), which indicates the selective cytotoxic effects in the MCF-7 cell line. Comparison of $IC_{50}$ values indicated that 40, 80, and 100% fractions after 48 hours of incubation significantly inhibited the growth of cancer cells, but the mechanism of cytotoxicity of these fractions is from the necrosis pathway. Therefore, in this cell line, 60% fraction is a suitable candidate for additional studies. Statistical analyzes in the SW872 cell line indicated that the viability of the most cytotoxic fraction with the lowest $IC_{50}$ values (60 and 80% fractions) was significantly different ($p < 0.0001$) with 40 and 100% fractions. Our finding showed that the 60 and 80% fractions inhibited cell growth by apoptosis induction pathway. In addition, the percentage of apoptotic cells in the 80% fraction is higher than the 60% fraction. The viability of 60 and 80% fractions are significantly different from DCM extract ($p < 0.05$) without depending on the time. A comparison of 80% fraction viability at both incubation times (24 and 48 h) showed that the cytotoxic effects of 80% fraction in SW872 cells compared to normal cells with a *p*-value less than 0.05 were selective. Thus, in total, 80% fraction is a better candidate for future research on liposarcoma cancer cells in future research. However, it is possible to obtain compounds that have selective cytotoxic effects on cancer cells by purifying the cytotoxic active ingredients from the 60% fraction. The results of the MTT test and statistical analysis showed that 60 and 80% fractions probably contain the most effective cytotoxic compounds against MCF-7 and SW872 cancer cells. The cytotoxic effects of these fractions are selectively on cancer cell lines with fewer adverse effects on the normal cell line. The essential oil also significantly inhibited cell growth. It has been demonstrated that the DCM extract of *A. turanica* had significant anti-cancer effects against K562 and HL-60 cell lines [47]. Two triterpenoid compounds called lopeol and lodartin were purified from *A. indica* as a cytotoxic active ingredient. These compounds inhibited the growth of breast cancer cells with $IC_{50}$ values of 28.08

and 25.18 μM [48]. The antioxidant and anti-cancer properties of *A. marschalliana* ethanolic extract were investigated and AGS trans phytol (29.22%), alpha-linoleic acid (13.47%), and n-hexadecanoic acid (9/28%) were reported as the effective cytotoxic compounds [49]. On the other hand, the anti-cancer effects of silver nanoparticles synthesized by *A. marschalliana* shoot against human gastrointestinal carcinoma were studied and flow cytometry results showed that the cytotoxic effects of silver nanoparticles with $IC_{50}$ are equivalent to 21.05 μg/mL due to induction of apoptosis [50]. Based on previous studies, the DCM extract of *Artemisia* has significant cytotoxic effects due to the presence of effective anti-cancer compounds. β-thujone and comphore were detected as the major compounds that the effective compounds according to the GC-MS analysis. Some of the antitumor agents extracted from Artemisia species include cesquiterpenalactones, terpenoids and flavonoids [34]. As previously mentioned, flow cytometry results illustrated that this extract leads the cell to apoptosis. Analysis of pro-apoptotic gene expression levels by RT-PCR showed that *A. marschalliana* extracts increased *P53* and *BAX* expression. In addition, the increase in BAX proteins level supported the RT-PCR data. These findings confirm the involvement of the innate pathway of cell death and suggest that *A. marschalliana* extracts induce apoptosis in cancerous cells. According to the findings, it can be concluded that 60 and 80% fractions of DCM extract of *A. marschalliana* can be a potential option for future studies in the field of identification and separation of pure cytotoxic compounds.

## Conclusion

Our results showed that the cytotoxic effects of *A. marschalliana* shoots are related to DCM extract. The 60% fraction has the highest effects of cytotoxicity by the mechanism of apoptosis in the MCF-7 cell line. It seems that this fraction has selective toxicity on cancer cells and can prohibit their cell growth, which increases with the incubation time. The viability of SW872 cells treated with 60 and 80% fractions of DCM extract was significantly reduced with other fractions. The main mechanism of cytotoxicity of 60 and 80% fractions in SW872 cells was the induction of apoptosis and 80% fraction selectively inhibited the growth of cancer cells. The essential oil of this plant also inhibited the growth of MCF-7 cancer cells. According to the above findings, it can be argued that the cell growth inhibitory compounds of DCM extract have accumulated in 60 and 80% fractions. Therefore, 60 and 80% fractions of DCM extract are the best candidates for future studies in the field of identification and separation of pure cytotoxic compounds.

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## Abbreviations

| | |
|---|---|
| RT-PCR | Real-Time Polymerase chain reaction |
| BMI | Body mass index |
| LS | Liposarcoma |
| n-Hex | n-Hexane |
| DCM | Dichloromethane |
| MeOH | methanol |
| MCF-7 cell | Human breast adenocarcinoma cell line |
| SW872 cell | Human liposarcoma cell line |
| MCF-10A cell | Normal human mammary epithelial cells |
| REC | Research Ethical Committee |
| DMSO | Dimethyl sulfoxide |
| DOS | Desoxycholic acid sodium salt |
| IC50 | Half-maximal inhibitory concentration |

## Authors' contributions

MZK and HF performed the experiments; PA and VT analyzed of data; MZK, VT, HF and PA prepared the manuscript; HF and VT wrote and edited the manuscript; PA designed and supervised the project. The author(s) read and approved the final manuscript.

## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Declarations

### Ethics approval and consent to participate

The experiments on plant extract were compiled and approved by the Research Ethical Committee (REC) Numbers: IR.TBZMED.RDC.1396.1187 and IR.TBZMED.RDC.1397.012, Tabriz University of Medical Sciences, Tabriz, Iran. The experimental research and field studies on plants, including the collection of plant material, comply with relevant institutional, national, and international guidelines and legislation.

### Consent for publication

Not applicable (NA).

### Competing interests

The authors declare no competing interests.

## References

1.  Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, et al. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin. 2016;66(4):271–89.
2.  Zarei A, Ashtiyani SC, Hamidizadeh S, Rezaei A. The study of the effects hydro-alcoholic extract of Eryngium billardieri on lipid profiles levels and liver and renal functions tests in hypercholesterolemic rats. Global J Pharmacol. 2015;9(1):21–7.
3.  Miller KD, Fidler-Benaoudia M, Keegan TH, Hipp HS, Jemal A, Siegel RL. Cancer statistics for adolescents and young adults, 2020. CA Cancer J Clin. 2020;70(6):443–59.
4.  Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin. 2021;71(1):7–33.
5.  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.
6.  Dodd LG. Update on liposarcoma: a review for cytopathologists. Diagn Cytopathol. 2012;40(12):1122–31.
7.  Conyers R, Young S, Thomas DM. Liposarcoma: molecular genetics and therapeutics. Sarcoma. 2010;2011:483154.
8.  Mousavi SM, Montazeri A, Mohagheghi MA, Jarrahi AM, Harirchi I, Najafi M, et al. Breast cancer in Iran: an epidemiological review. Breast J. 2007;13(4):383–91.
9.  Taraphdar AK, Roy M, Bhattacharya R. Natural products as inducers of apoptosis: Implication for cancer therapy and prevention. Curr Sci. 2001: 80(11):1387–96.
10. Fulda S, Efferth T. Selected secondary plant metabolites for cancer therapy. World J Tradit Chin Med. 2015;1(1):24.
11. Hosseini K, Jasori S, Delazar A, Asgharian P, Tarhriz V. Phytochemical analysis and anticancer activity of Falcaria vulgaris Bernh growing in Moghan plain, northwest of Iran. BMC Complement Med Ther. 2021;21(1):1–10.
12. El-Massry KF, El-Ghorab AH, Farouk A. Antioxidant activity and volatile components of Egyptian Artemisia judaica L. Food Chem. 2002;79(3):331–6.
13. Tan RX, Zheng W, Tang H. Biologically active substances from the genus Artemisia. Planta Med. 1998;64(04):295–302.
14. Woerdenbag HJ, Moskal TA, Pras N, Malingré TM, El-Feraly FS, Kampinga HH, et al. Cytotoxicity of artemisinin-related endoperoxides to Ehrlich ascites tumor cells. J Nat Prod. 1993;56(6):849–56.
15. Efferth T, Olbrich A, Bauer R. mRNA expression profiles for the response of human tumor cell lines to the antimalarial drugs artesunate, arteether, and artemether. Biochem Pharmacol. 2002;64(4):617–23.
16. Sarath VJ, So C-s, Won YD, Gollapudi S. Artemisia princeps var orientalis induces apoptosis in human breast cancer MCF-7 cells. Anticancer Res. 2007;27(6B):3891–8.
17. Hu YQ, Tan RX, Chu MY, Zhou J. Apoptosis in human hepatoma cell line SMMC-7721 induced by water-soluble macromolecular components of Artemisia capillaris Thunberg. Jpn J Cancer Res. 2000;91(1):113–7.
18. Kim K-N, Lee J-A, Yoon W-J, Kim J-Y, Song G-P, Park S-Y. The cytotoxicity of Artemisia fukudo extracts against HL-60 cells. J Korean Soc Food Sci Nutr. 2007;36(7):819–24.
19. Forouhandeh H, Rezaei Param Z, Molavi O, Asgharian P, Tarhriz V. Evaluation of anti-proliferative activity of Eryngium caucasicum on melanoma cancer cells. BMC Complement Med Ther. 2022;22(1):1–9.
20. Mollaei S, Sedighi F, Habibi B, Hazrati S, Asgharian P. Extraction of essential oils of Ferulago angulata with microwave-assisted hydrodistillation. Ind Crop Prod. 2019;137:43–51.
21. El-Aarag BY, Kasai T, Zahran MA, Zakhary NI, Shigehiro T, Sekhar SC, et al. In vitro anti-proliferative and anti-angiogenic activities of thalidomide dithiocarbamate analogs. Int Immunopharmacol. 2014;21(2):283–92.
22. Hasanbeiglu S, Hosseini K, Molavi O, Asgharian P, Tarhriz V. Eryngium billardieri extract and fractions induces apoptosis in cancerous cells. Anti Cancer Agents Med Chem. 2021;22(11):2189–201.
23. Levine CB, Bayle J, Biourge V, Wakshlag JJ. Cellular effects of a turmeric root and rosemary leaf extract on canine neoplastic cell lines. BMC Vet Res. 2017;13(1):1–12.
24. El-Aarag B, Kasai T, Masuda J, Agwa H, Zahran M, Seno M. Anticancer effects of novel thalidomide analogs in A549 cells through inhibition of vascular endothelial growth factor and matrix metalloproteinase-2. Biomed Pharmacother. 2017;85:549–55.
25. Devarajan E, Sahin AA, Chen JS, Krishnamurthy RR, Aggarwal N, Brun A-M, et al. Down-regulation of caspase 3 in breast cancer: a possible mechanism for chemoresistance. Oncogene. 2002;21(57):8843–51.
26. Tarhriz V, Eyvazi S, Musavi M, Abasi M, Sharifi K, Ghanbarian H, et al. Transient induction of Cdk9 in the early stage of differentiation is critical for myogenesis. J Cell Biochem. 2019;120(11):18854–61.
27. Fathi E, Farahzadi R, Vietor I, Javanmardi S. Cardiac differentiation of bone-marrow-resident c-kit+ stem cells by L-carnitine increases through secretion of VEGF, IL6, IGF-1, and TGF-β as clinical agents in cardiac regeneration. J Biosci. 2020;45(1):1–11.
28. Ninfa AJ, Ballou DP, Benore M. Fundamental laboratory approaches for biochemistry and biotechnology. Hoboken: Wiley; 2009.
29. Fathi E, Vietor I. Mesenchymal stem cells promote caspase expression in Molt-4 leukemia cells via GSK-3α/and ERK1/2 signaling pathways as a therapeutic strategy. Curr Gene Ther. 2021;21(1):81–8.

30. Bagheri Y, Barati A, Nouraei S, Namini NJ, Bakhshi M, Fathi E, et al. Comparative study of gavage and intraperitoneal administration of gamma-oryzanol in alleviation/attenuation in a rat animal model of renal ischemia/reperfusion-induced injury. Iran J Basic Med Sci. 2021;24(2):175.

31. Asnaashari S, Afshar FH, Ebrahimi A, Moghadam SB, Delazar A. Chemical composition and radical scavenging activity of essential oil and methanolic extract of Eremostachys azerbaijanica Rech. f. from Iran. Res Pharm Sci. 2016;11(2):113.

32. Hallaj-Nezhadi S, Hamdipour R, Shahrvirani M, Chapeland-leclerc F, Ruprich-Robert G, Esnaashari S, et al. Antimicrobial activity of Bacillus sp. isolated strains of wild honey. BMC Complement Med Ther. 2022;22(1):1–12.

33. Ngoma T. World Health Organization cancer priorities in developing countries. Ann Oncol. 2006;17:viii9–viii14.

34. Fletcher CD, Unni KK, Mertens F. Pathology and genetics of tumours of soft tissue and bone: Iarc. Geneve: World Health Organization; 2002.

35. Reed D, Altiok S. Metastatic soft tissue sarcoma chemotherapy: an opportunity for personalized medicine. Cancer Control. 2011;18(3):188–95.

36. Prakash O, Kumar A, Kumar P. Anticancer potential of plants and natural products. Am J Pharmacol Sci. 2013;1(6):104–15.

37. Nirmala MJ, Samundeeswari A, Sankar PD. Natural plant resources in anticancer therapy-a review. Res Plant Biol. 2011;1(3):01–14.

38. Asgharian P, Quispe C, Herrera-Bravo J, Sabernavaei M, Hosseini K, Forouhandeh H, et al. Pharmacological effects and therapeutic potential of natural compounds in neuropsychiatric disorders: an update. Front Pharmacol. 2022;13.

39. Greenwell M, Rahman P. Medicinal plants: their use in anticancer treatment. Int J Pharm Sci Res. 2015;6(10):4103.

40. Forouhandeh H, Zarchini M, Safarzadeh E, Molavi O, Asgharian P, Tarhriz V. Evaluation of the cytotoxic activity of Anthriscus nemorosa on breast Cancer cells. J Contemp Med Sci. 2021;7(4):217–26.

41. Mousavi B, Tafvizi F, Zaker BS. Green synthesis of silver nanoparticles using Artemisia turcomanica leaf extract and the study of anti-cancer effect and apoptosis induction on gastric cancer cell line (AGS). Artif Cells Nanomed Biotechnol. 2018;46(sup1):499–510.

42. Rahimivand M, Tafvizi F, Noorbazargan H. Synthesis and characterization of alginate nanocarrier encapsulating Artemisia ciniformis extract and evaluation of the cytotoxicity and apoptosis induction in AGS cell line. Int J Biol Macromol. 2020;158:338–57.

43. Taleghani A, Emami SA, Tayarani-Najaran Z. Artemisia: a promising plant for the treatment of cancer. Bioorg Med Chem. 2020;28(1):115180.

44. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35(4):495–516.

45. Taghizadeh Rabe SZ, Mahmoudi M, Ahi A, Emami SA. Antiproliferative effects of extracts from Iranian Artemisia species on cancer cell lines. Pharm Biol. 2011;49(9):962–9.

46. Aslany S, Tafvizi F, Naseh V. Characterization and evaluation of cytotoxic and apoptotic effects of green synthesis of silver nanoparticles using Artemisia Ciniformis on human gastric adenocarcinoma. Mater Today Commun. 2020;24:101011.

47. Tayarani-Najaran Z, Sareban M, Gholami A, Emami SA, Mojarrab M. Cytotoxic and apoptotic effects of different extracts of Artemisia turanica Krasch. On K562 and HL-60 cell lines. Sci World J. 2013;2013:628073.

48. Zeng YT, Jiang JM, Lao HY, Guo JW, Lun YN, Yang M. Antitumor and apoptotic activities of the chemical constituents from the ethyl acetate extract of Artemisia indica. Mol Med Rep. 2015;11(3):2234–40.

49. Salehi S, Mirzaie A, Sadat Shandiz SA, Noorbazargan H, Rahimi A, Yarmohammadi S, et al. Chemical composition, antioxidant, antibacterial and cytotoxic effects of Artemisia marschalliana Sprengel extract. Nat Prod Res. 2017;31(4):469–72.

50. Salehi S, Shandiz SAS, Ghanbar F, Darvish MR, Ardestani MS, Mirzaie A, et al. Phytosynthesis of silver nanoparticles using Artemisia marschalliana Sprengel aerial part extract and assessment of their antioxidant, anticancer, and antibacterial properties. Int J Nanomedicine. 2016;11:1835.

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