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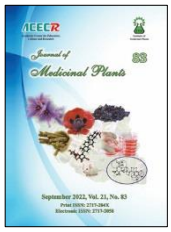

Research Article

Bioassay-guided isolation of phenolic apoptosis inducer anticancer agents from *Berberis vulgaris* fruits

Sobhan Ghafourian[1], Ehsan Ghafourian[2], Seyed Mohammad Zarei[3,*], Fereshteh Jalilian[4], Leila Hosseinzadeh[4], Zulfiqar Ali[5], Yan-Hong Wang[5], Yalda Shokoohinia[6]

[1] *Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran*
[2] *Students Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran*
[3] *Department of Pharmacognosy and Traditional Pharmacy, School of pharmacy, Baqiyatallah University of Medical Sciences, Tehran, Iran*
[4] *Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran*
[5] *National Center for Natural Products Research, University of Mississippi, Oxford, MS, USA*
[6] *Ric Scalzo Botanical Research Institute, Southwest College of Naturopathic Medicine, Tempe, AZ, USA*

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ABSTRACT

Background: Cancer development is a multi-stage process in which apoptosis plays an important role. Apoptotic agents from natural products like phenolic compounds can be used effectively in the treatment of cancer. *Berberis vulgaris* L. fruits are a rich source of natural phenolic compounds and have been evaluated for their apoptotic effects. **Objective:** this study was carried out to evaluate the apoptotic effect of *B. vulgaris* fruits in normal and cancerous cells by investigating p53, Bax, and Bcl-2 genes expression and identifying an active compound. **Methods:** *B. vulgaris* fruits were extracted with ethanol using maceration method. To yield an active compound, the extract was divided into fractions and subfractions that could be evaluated using chromatography techniques, and the purified compound structure was determined by nuclear magnetic resonance (NMR). The cytotoxic activity of the selected fraction against 3T3 and MCF-7 cell lines along with its effect on the mRNA apoptosis regulating genes like p53, Bax, and Bcl-2 were investigated subsequently. **Results:** Results showed that *B. vulgaris* extract increased the mRNA level of p53 and Bax and decreased Bcl-2 mRNA level in cancer cell lines. The structure of the purified compound was determined as the 3-caffeoylquinic acid (chlorogenic acid). **Conclusion:** *B. vulgaris* fruit has *in vitro* antineoplastic activity by stimulating cellular apoptosis via increasing the levels of p53 and Bax proteins and decreasing Bcl-2 and comprises chlorogenic acid as one of its phenolic phytochemicals.

Abbreviations: mRNA, messenger Ribonucleic Acid; HPLC, High-Performance Liquid Chromatography; TLC, Thin layer Chromatography; NMR, Nuclear Magnetic Resonance; CC, Column Chromatography; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; IC_{50}, Half-Maximal Inhibitory Concentration; DMSO, Dimethyl sulfoxide; ELISA, Enzyme-Linked Immunosorbent Assay; DOX, Doxorubicin; PCR, Polymerase Chain Reaction; cDNA, Complementary Deoxyribonucleic Acid; ^{1}H-NMR, Proton Nuclear Magnetic Resonance; ^{13}C-NMR, Carbon-13 Nuclear Magnetic Resonance; BB, Broad Bond; COSY, Correlated Spectroscopy; HMBC, Heteronuclear Multiple Bond Correlation

*Corresponding author: sm.zarei@bmsu.ac.ir
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1. Introduction

Cancer is currently the second leading cause of death worldwide, killing millions each year. Cancer deaths are estimated to increase to 13 million by 2030 [1, 2].

Therefore, in recent years, more attention is given to finding new cancer treatments. In general, cancer development is a multi-stage and complex process based on which various treatments have been evaluated. Apoptosis is one of the processes that has received much attention for decreasing the incidence and treatment of cancer. Apoptosis, programmed cell death type 1, is a pivotal phenomenon in tissue homeostasis which results in removing cells that the body no longer needs [3]. Control of this process is influenced by the expression of regulatory genes such as p53 tumor inhibitor, Bax regulator, and anti-apoptotic Bcl-2 [4]. It is important to consider the role of apoptosis in preventing diseases such as cancer in which cell proliferation occurs uncontrollably. Therefore, understanding the mechanisms of apoptosis and identifying the compounds that induce this process can be useful in the treatment of cancer [1].

In pharmaceutical research, herbs have received much attention as naturally rich sources of new therapeutic agents. Accordingly, phenolic compounds are a group of plant secondary metabolites that are produced by plants in response to their ecological or physiological needs and act as antioxidants, attractants, ultraviolet screens, signal compounds, and defense response chemicals [5]. Also, these compounds in humans are involved in various processes such as anti-aging, immunological defense, anti-inflammatory, and antioxidant activities, as well as anti-proliferative activities that can be considered as appropriate agents to regulate programmed cell death. In addition, numerous studies have shown that phenolic compounds play an effective role in the prevention of chronic disease [5, 6]. Therefore, plants that are rich in these compounds are of special importance.

Berberis vulgaris L. (Berberidaceae) is a rich source of phenols and based on its medicinal properties its fruit has been used in traditional Iranian medicine for centuries to treat liver diseases, eye sores, toothache, respiratory problems like asthma, skin disorders like pigmentation and removing oral and topical swellings and inflammations [7-9]. Therefor in this study fruits of *B. vulgaris* as a source of bioactive phenolic compounds were selected to evaluate its effect on apoptosis and the level of expression of some genes that control this process be evaluated.

Previously, we had reported that the ethanolic extract of *B. vulgaris* fruits induces apoptosis in breast cancer cell line, MCF-7 [10]. But, this study aimed to evaluate the rate of apoptosis in 3T3 normal cells compared to MCF-7 cancerous cells using *B. vulgaris* fruits extract. Also, the molecular mechanism of apoptosis was investigated by measuring the mRNA level of some genes involved in the apoptotic process: p53, Bax, and Bcl-2. The active molecule in the extract that leads to apoptosis was also examined and identified.

2. Materials and Methods

2.1. Chemicals

The graded solvents for high-performance liquid chromatography (HPLC) were purchased from Merck, Germany. The extraction solvents were prepared by Dr. Mojallali Company, I.R. Iran.

Chromatography procedures were performed using reversed-phase silica gel 0.015-0.030 µm

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(Merck) and thin-layer chromatography (TLC) plates (silica gel GF_{254} plates, 20 × 20 cm, 0.5 mm, Merck). A Young Lin apparatus, equipped with an YL 9111S pump and YL 9160 PDA detector was used with a Vertica Reverse phase RP18 250x 30 mm column with 10 ml/min flow rate were used to perform a preparative HPLC. A 500 MHz Bruker spectrometer using D_2O and MeOD as solvents was used to record the NMR spectra.

2.2. Plant material

Fruits of *B. vulgaris*, were obtained from Birjand market in November 2018 and identified by Mohsen Pouyan, plant taxonomist of Birjand University. The taxonomy of the plant was compared with specimen No. KSh 8031 at Razi University herbarium in Kermanshah, I.R. Iran.

2.3. Extraction

B. vulgaris fruits were shade dried and ground into a powder (200 g). The powder was macerated in 1000 ml of ethanol 70 % at room temperature. The macerating process was done three times (every 24 hours) and the solution was shaken 3 minutes every day.

The ethanolic solution was filtered and evaporated under vacuum condition by a rotary evaporator to obtain the whole extract. An aliquot of extract (25 g, 1A) was subjected to a column of reversed-phase silica gel (125 g) and eluted with a mixture of water/ethanol (90:10 to 10:90 v/v). Applying TLC technique and cerium sulfate/molybdate as a phytochemical reagent five different fractions (2A-E) collected and were used for the next steps.

Then, 2B fraction (436.6 mg) was purified by reversed-phase column chromatography (CC) (water/ethanol; 70:30 to 10:90 v/v) to render five sub-fractions (7A-E). For more purification, 7A sub-fraction (35 mg), as an effective sub-fraction in biological tests, was injected into HPLC using a reversed-phase column and the mobile phase of water/methanol (80:20 v/v) to obtain fraction 11D containing pure compound **1**.

2.4. Cell culture and viability assay

3T3 cells lines were utilized as normal cells and MCF-7 cell lines were used as the cancerous cells. The cells lines were provided by Dr. Nasser Abbasi of the Herbal Medicine Research Center, Ilam University of Medical Sciences in I.R. Iran. A standard protocol was used to culture the cells [11], and to determine the cytotoxic effect of the fractions (2A-E). Two methods including Trypan blue dye exclusion and MTT assay were applied to determine the IC_{50} of the fractions. Briefly, different concentrations (1, 5, 10, 15, and 50 µg/ml) of each fraction were used for cytotoxicity evaluation. These concentrations were added to the cell cultures and after 24 hours cytotoxicity was evaluated. For determination of toxicity by Trypan blue, 50 µl of cell suspension was added to a micro-vial, then, 50 µl of Trypan blue dye (4 %) was added and gently mixed. After the mixture was placed at room temperature for 2 minutes, cell observation was done by the hemocytometer slide. Cell viability was calculated with this formula:

$$\% \text{ viable cells} = [1.00 - (\text{Number of blue cells} / \text{Number of total cells})] \times 100$$

In addition, the MTT assay was applied to all concentrations based on the manufacturer's protocol. MTT solution (USB Corporation, USA) was added to each well and the plates were incubated for 4 h at 37 °C. MTT solution was removed and DMSO was added to dissolve formazan crystals. The absorbance was read at 570 nm using the microplate ELISA reader

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(Bio-Rad Laboratories, USA) [10]. Cell viability percentage was calculated using the following formula:

(%) = (Abs $_{Treatment}$ /Abs $_{Control}$) × 100

The results were compared with doxorubicin (DOX) as a positive control. Prism 6 software obtained the results of the IC_{50} of the extract against 3T3 and MCF-7 cells.

2.5. Real-time quantitative PCR

2.5.1. RNA Extraction

Manufacturer instructions for the RNA extraction kit (GeneAll, South Korea) were used to extract RNA for real-time PCR. DNase I was used for the elimination of DNA during RNA extraction. A spectrophotometer (NanoDrop, Eppendorf, Germany), measuring maximum absorbance at 260 nm, was used to quantify the extracted RNA.

The SCRIPT cDNA Synthesis kit (Jena Bioscience, Germany) synthesized the complementary DNA (cDNA).

2.5.2. Qualitative PCR assay

GenScript software designed the specific primers for the p53, Bax, and Bcl-2 genes and they were synthesized by Sigma (USA). A qualitative PCR assay was used to evaluate the ability of the specific gene primer (Table 1) to amplify the appropriate amplicon length, using 4 µl of cDNA and 1 nM concentration of each primer in a total volume of 20 µl per reaction in a PCR master mix. The thermocycling conditions consisted of an initial denaturation for 15 min at 95 ºC, followed by 40 cycles of 95 ºC for 15 seconds, 60 °C for 40 seconds and 68 ºC for 20 seconds. All reactions were performed using an Eppendorf thermocycler (Germany).

2.6. Quantitative real-time PCR

Quantitative PCR assay was done using 4 µl of cDNA and 1 nM concentration of each primer in a total volume of 20 µl per reaction in a 2x QuantiFast SYBR Green RT-PCR master mix. The cycling conditions were as follows: one cycle of 95 ºC for 15 min as the holding time and then 95 ºC for 15 seconds (denaturation), 60 °C for 40 min (annealing/extension), and 68 ºC for 20 seconds as final extension. Forty cycles as a cycle cut-off point for no detection was set and β-actin was used as calibrator control. Then, the results were analyzed quantitatively to estimate the mRNA level of p53, Bax, and Bcl-2 genes in treated 3T3 and MCF-7 cells by the comparative threshold cycle (CT) method using the formula $2^{-\Delta\Delta(CT)}$.

Table 1. Primers sequence in qPCR

Name	Sequence (5'-3')	Strand	Tm °C	Purification method
Primer BCL-2: Amplicon Size = 122				
query_L1	ATGTGTGTGGAGAGCGTCAACC	Forward	58.85	HPLC
query_R1	GCATCCCAGCCTCCGTTATC	Reverse	58.96	HPLC
Primer p53: Amplicon, Size = 145				
query_L1	CTGAGGTTGGCTCTGACTGTACCACCATCC	Forward	59.00	HPLC
query_R1	CTCATTCAGCTCTCGGAACATCTCGAAGCG	Reverse	58.16	HPLC
Primer Bax: Amplicon, Size = 148				
query_L1	CCTTTTCTACTTTGCCAGCAAAC	Forward	59.00	HPLC
query_R1	GAGGCCGTCCCAACCAC	Reverse	58.16	HPLC

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2.7. Statistical analysis

Statistical analyses were performed using SPSS software (v 19.0; IBM New York, NY, USA). A Student's t-test was performed when statistical differences were detected. Data are reported as mean ± SD at a significance level of $P < 0.05$.

3. Results

3.1. Cytotoxicity assay

The IC_{50} concentration of ethanolic extract of *B. vulgaris* and its fractions against normal cells (3T3) and cancerous cells (MCF-7) are reported in Table 2. The results showed the prepared extract and all the fractions were potent and were able to kill more than 50 % of cancerous cells and IC_{50} against MCF-7 cells was successfully reached. On the other hand, these samples were also toxic for normal 3T3 cells. Therefore, it is important to select a fraction that is safe for normal cells and exerts the most significant cytotoxic effect on cancer cells.

The results of cytotoxicity by Trypan blue and MTT assay showed that fraction 2B at 10 µg/m had the highest toxic effect against cancer cells and can kill about 50 % of cancer cells and was safe for normal cells. Because of these reasons, fraction 2B was selected as the most bioactive fraction for further cell-based analyses and also was divided into different sub-fractions (7A-E) to phytochemical analysis.

3.2. Purification of active compound using HPLC

Considering biological tests, effective sub-fraction (the 7A sub-fraction) was injected into HPLC using a reversed-phase column to obtain purified active compound. The pure compound structure was determined by NMR as the 3-caffeoylquinic acid (16 mg) (Chlorogenic acid; $C_{16}H_{18}O_9$) (Fig. 1).

Table 2. The IC_{50} (µg/ml) of *B. vulgaris* fruit extract fractions against 3T3 and MCF-7 cells.

Fractions	IC_{50}	
	3T3 cells	MCF-7 cells
2A	20	35
2B	25	9
2C	20	35
2D	20	15
2E	15	15
2F	40	50

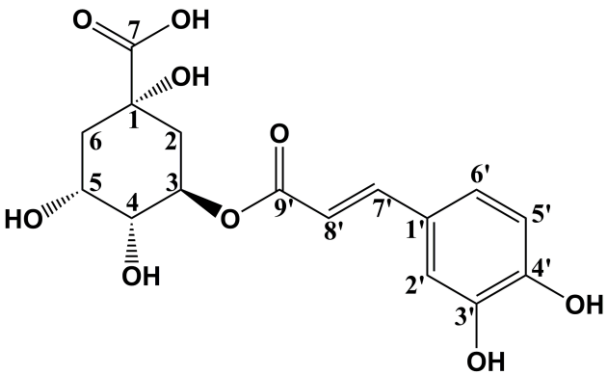

Fig. 1. Structure of Chlorogenic acid

The analysis of structure based on NMR is presented below:

$C_{16}H_{18}O_9$; MW 354.1 g/mol; ^{1}H-NMR (D_2O + MeOD, 500 MHz, *J*in Hz): δ_H 2.13-2.21 (*m*, 2H, H-2_a and H-2_b), 5.30 (*t*, *J*= 10, 1H, H-3), 3.82 (*dd*, *J* = 10, 3.5, 1H, H-4), 4.22 (*d*, *J* = 3.5, 1H, H-5), 1.98-2.11 (*m*, 2H, H-6_a and H-6_b), 7.12 (*d*, *J* = 2, 1H, H-2'), 6.87 (*d*, *J* = 8, 1H, H-5'), 7.04 (*dd*, *J* = 8, 2, 1H, H-6'), 7.57 (*d*, *J* = 16, 1H, H-7'), 6.32 (*d*, *J* = 16, 1H, H-8'). ^{13}C-NMR (D2O + MeOD, 125 MHz): δ_C 77.0 (*s*, C-1), 38.8 (*t*, C-2), 72.1 (*d*, C-3), 73.3 (*d*, C-4), 71.3 (*d*, C-5), 38.0 (*t*, C-6), 179.8 (*s*, C-7), 127.8 (*s*, C-1'), 115.6 (*d*, C-2'), 145.7 (*s*, C-3'), 148.5 (*s*, C-4'), 116.9 (*d*, C-5'), 123.5 (*d*, C-6'), 147.2 (*d*, C-7'), 115.2 (*d*, C-8'), 169.6 (*s*, C-9').

3.3. The effect of the selected fraction on expression levels of apoptosis-regulatory genes

After ensuring the promising cytotoxic effect of selected fraction (7A) on the MCF-7 cancer cells, further analyses have been done on this active fraction using a one-step real-time PCR assay to quantify the mRNA levels of well-known apoptosis-regulatory genes, p53, Bax, and Bcl-2. For this purpose, the IC_{50} concentration of fraction (10 µg/ml) against MCF-7 cells, which was non-toxic for 3T3 normal cells, was selected for molecular analyses. Furthermore, to provide a comparative analysis between the fraction and DOX, as one of the most potent anticancer medicaments, we used this agent as control. The results corresponding to the mRNA level of p53 gene in 3T3 and MCF-7 cells, before and after treatment, have been illustrated in Fig. 2. According to the results, the p53 expression in MCF-7 cells increased after treatment with selected fraction (7A) at IC_{50} concentration. Similar results have been observed for DOX-treated cells, however, it was more toxic than the fraction.

The mRNA levels of Bax and Bcl-2 genes have also been presented in Fig. 3 and 4. The results show that the selected fraction effectively up-regulated expression of the pro-apoptotic Bax gene and down-regulated the anti-apoptotic Bcl-2 gene expression in MCF-7 cancer cells. Likewise, cells treated with DOX can show a similar trend. However, the selected fraction could increase the level of Bax expression more than DOX. On the other hand, there was no significant difference between the effects of these agents on the mRNA level of the anti-apoptotic Bcl-2 gene in MCF-7 cells. In addition to the evaluation of changes in Bax and Bcl-2 expression level, determining the expression ratio of the Bax/Bcl-2 genes is also important. The results showed that after treating the cells with selected fraction and DOX, the amount of this ratio was increased in MCF-7 compared to 3T3. The highest level of this ratio in cancer cells compared to normal cells indicates the more significant effect of the fraction in the apoptosis induction in the cancer cells. The results of statistical analysis of p53 showed that there was a significant difference between fraction and DOX as well as the control sample ($P \leq 0.05$). Regarding Bcl-2 gene expression, no significant difference was observed between DOX and fraction ($P \geq 0.05$), however, there was a significant difference with normal cell expression ($P \leq 0.05$). The results of statistical analysis of Bax showed a significant difference between fraction and DOX as well as the control sample ($P \leq 0.05$).

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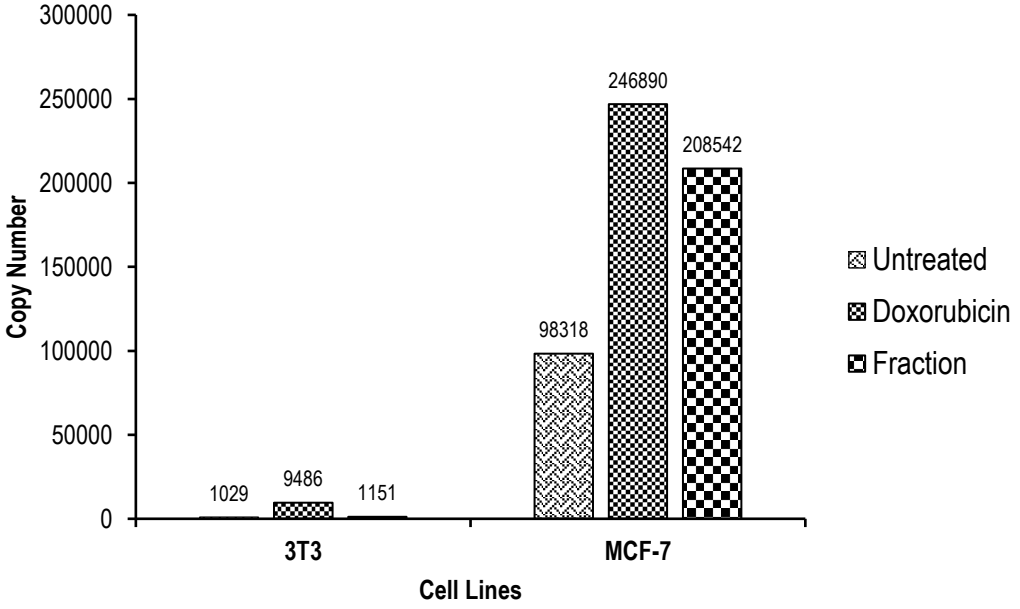

Fig. 2. The level of *p53* gene expression before and after treatment with selected fraction.

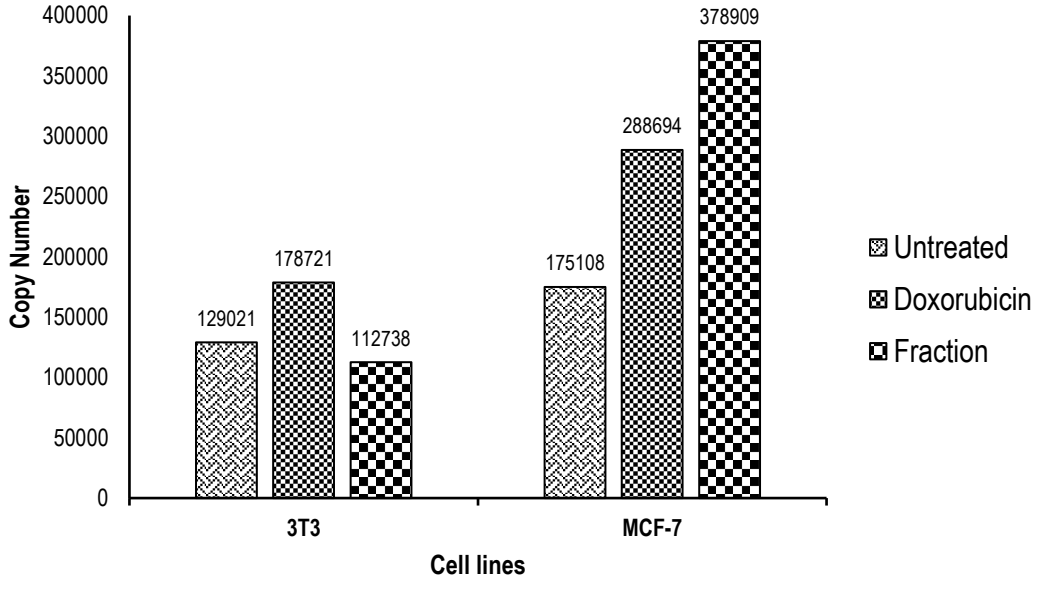

Fig. 3. The level of *Bax* gene expression before and after treatment with selected fraction.

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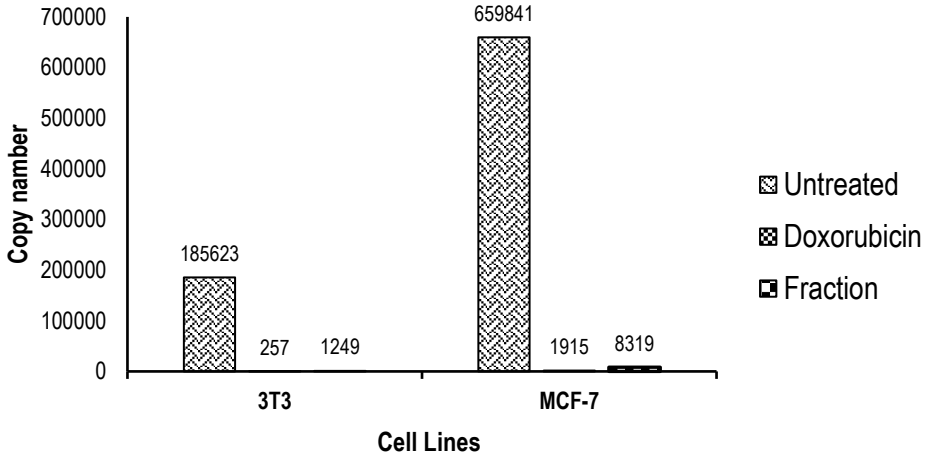

Fig. 4. The level of *Bcl-2* gene expression before and after treatment with selected fraction.

4. Discussion

4.1. Structure elucidation

Investigation of the ^{1}H-NMR and ^{13}C-NMR spectra shows signals in down and upfield areas that reveal the presence of aliphatic, olefinic, and aromatic structures in the purified compound. Based on Q-DEPT and broad bond (BB) decoupled ^{13}C-NMR, compound **1** contains two methylenes (δ_C 38.8, 38.0), eight methines (δ_C 147.2, 123.5, 116.9, 115.6, 115.2, 73.3, 72.1, 71.3) as well as six quaternary carbons (δ_C 179.8, 169.6, 148.5, 145.7, 127.8, 77.0) in which two carbons (δ_C 179.8, 169.6) appear in the downfield region which are signs of ester or carboxylic functional groups. ^{13}C NMR BB along with COSY and HMBC experiments (Fig. 5 and 6) indicate an olefinic bond involving carbons resonated at 147.2 and 115.2 which are in resonance with protons that appear in δ_H 7.57 (*d*) and 6.32 (*d*), respectively. Based on these data and their high coupling constant (J = 16) their trans stereochemistry could be concluded. The HMBC spectrum demonstrated that one of the olefinic carbons (δ_C 147.2) is connected to an aromatic spin system involving 6 carbons, two of them are oxygenated, whereas another olefinic carbon (δ_C 115.2) links to an ester functional group. Taking this arrangement of phenyl group, alkene bond and ester functional group along with a literature review [12, 13] indicate the presence of caffeoyl ester moiety in compound **1**. Without caffeoyl ester moiety, based on COSY and HMBC spectra, remaining carbons which comprise a carboxylic acid function (δ_C 179.9), a quaternary carbon (δ_C 77.0), 2 methylenes (δ_C 38.8 & 38.0) and 3 methines (δ_C 73.3, 72.1, 71.3), compose an aliphatic 6 membered carbon ring that attaches to a carboxylic acid group. Based on BB ^{13}C-NMR, quaternary carbon and all methine carbons are oxygenated which are assigned to C-1, C-4, C-3, and C-5, respectively. Methylenes (δ_C 38.8, 38.0) are assigned to C-2 and C-6 positions. Also, HMBC shows the aliphatic ring links to carboxylic acid function (δ_C 179.9) in C-1 (δ_C 77.0) position. Literature review [12, 13] and existing data confirmed the structure of quinic acid, which based on HMBC spectrum joined to a caffeoyl group by C-3. Finally, considering all the above-mentioned data and analysis of references [12, 13], compound 1 was determined to be 3-caffeoylquinic acid (chlorogenic acid).

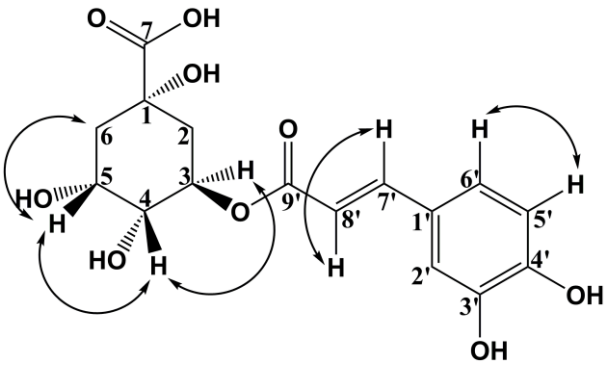

Fig. 5. H-H COSY correlations of Chlorogenic acid

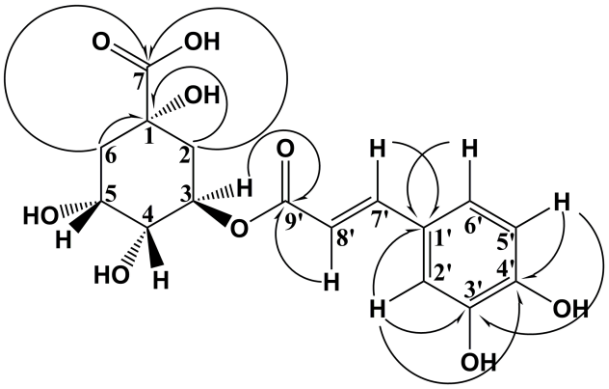

Fig. 6. HMBC correlations of chlorogenic acid

4.2. Cell culture studies

The findings presented in this study demonstrated the prominent anti-proliferative effect of *B. vulgaris* fruits ethanolic extract and its fractions against MCF-7 cancer cells. The ethanolic extract of *B. vulgaris* has been previously subjected to cytotoxic studies, which exhibited promising anti-proliferative and anti-tumor effects on different types of cancer cells [14]. The prominent cytotoxic effect of this extract is considered due to its content of active phytochemicals [15]. So, it has been considered as one of the major aims of this study to examine this assumption. For this purpose, the inhibitory effect of the extract fractions was investigated.

Based on the fact that non-selective chemotherapy can, unfortunately, result in systemic toxicity, the toxicity of these samples against normal cells (3T3) has been conducted as well. The non-toxic concentration (against normal 3T3 cells) of the most active fraction was further analyzed to assure how this fraction exerts the cytotoxic effect and whether it can manipulate the mechanism of apoptosis or not. The induction of apoptosis was confirmed by determining the variation of the mRNA levels of p53, Bax, and Bcl-2 genes, as three apoptosis-regulatory genes, before and after treatment, and the results were compared to the corresponding results of DOX. Quantitative evaluation of mRNA levels in MCF-7 cells treated with the

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selected fraction showed an increase in the mRNA levels of the p53 tumor suppressor gene and the Bax to Bcl-2 mRNA ratio due to the increase in Bax expression and significant down-regulation of Bcl-2 expression ($P \leq 0.05$). Previous studies report similar results [10], in which MCF-7 cells treated with ethanolic extract of *B. vulgaris* underwent apoptosis cell death associated with inhibition of the anti-apoptotic Bcl-2 mRNA [10].

The p53 gene is one of the major factors responsible for the regulation of the Bax and Bcl-2 protein expressions in response to DNA damage and triggering a variety of cell cycle regulatory events [16]. The results of the present study exhibited that the most active fraction of *B. vulgaris* fruits extract causes apoptosis of MCF-7 cells in a p53-dependent pathway by increasing the level of p53 mRNA in cancerous cells. Bax and Bcl-2 are two other apoptosis-regulatory genes and the ratio between them is a decisive factor regarding the potential of chemopreventive therapeutics to induce apoptosis in tumor cells. In the current study, the expression of Bax and Bcl-2 genes was affected by the most active fraction resulting in a rise in the mRNA level of Bax and a decrease in Bcl-2 mRNA level. In addition, the results showed that the expression of the p53 and Bax genes in MCF-7 cancer cells is significantly higher than 3T3 normal cells, which indicates a greater effect of the selected fraction against cancer cells ($P \leq 0.05$).

Regarding identification of chlorogenic acid as the major compound of effective subfraction, it indicates chlorogenic acid ability to causes apoptosis of MCF-7 cells in a p53-dependent pathway. This event is carried out by an increase of p53 and Bax genes expression and reduction of Bcl-2 gene expression. These findings are in accordance to previous studies reported chlorogenic acid induces apoptosis however other mechanisms like inhibition of MMP-9, reactive oxygen species production increase, reduction of mitochondrial membrane potential, and increasing the activation of caspase-3 pathways have been involved [17-19].

5. Conclusion

Finally, our results confirm the ability of the selected fraction of *B. vulgaris* fruit extract to induce apoptosis, which is also demonstrated by the molecular analyses associated with apoptosis-regulatory genes. Also, the apoptotic effect of this fraction was found to be as high as that of DOX (a well-known marketed medicament for the treatment of breast cancer) and a prominent portion of this activity is related to chlorogenic acid.

Author contributions

E. G. and F. J. contributed in collecting plant sample and identification, running the laboratory work, and contributed to chromatographic analysis. L. H., S. M. Z., Y. H. W and Z. A. helped with data analysis. S. G. and Y. S. designed the study, supervised the laboratory work. Y. S. and S. M. Z drafted the paper. S. G. contributed to biological studies. Z. A. and Y. H. W. contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflict of interest

The authors declare that there is no conflict of interest.

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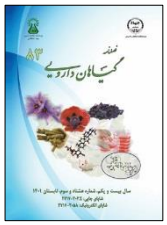

مقاله تحقیقاتی

جداسازی ترکیب‌های فنولی ضدسرطان القاکننده آپوپتوز از میوه‌های گیاه زرشک با کمک روش سنجش زیستی

سبحان غفوریان[۱]، احسان غفوریان[۲]، سیدمحمد زارعی[۳،*]، فرشته جلیلیان[۴]، لیلا حسین‌زاده[۴]، ذوالفقار علی[۵]، یان‌هونگ وانگ[۵]، یلدا شکوهی‌نیا[۶]

[۱] مرکز تحقیقات میکروب‌شناسی بالینی، دانشگاه علوم پزشکی ایلام، ایلام، ایران
[۲] کمیته تحقیقات دانشجویی، دانشگاه علوم پزشکی کرمانشاه، کرمانشاه، ایران
[۳] گروه فارماکوگنوزی و داروسازی سنتی، دانشکده داروسازی، دانشگاه علوم پزشکی بقیه‌الله(عج)، تهران، ایران
[۴] مرکز تحقیقات علوم دارویی، پژوهشکده سلامت، دانشگاه علوم پزشکی کرمانشاه، کرمانشاه، ایران
[۵] مرکز ملی تحقیقات ترکیبات طبیعی، دانشگاه می‌سی‌سی‌پی، آکسفورد، ایالت می‌سی‌سی‌پی، آمریکا
[۶] پژوهشکده گیاهان دارویی ریک اسکالزو، دانشگاه طب طبیعی جنوب غرب، تمپی، ایالت آریزونا، آمریکا

اطلاعات مقاله

گل‌واژگان:
آپوپتوز
زرشک
سرطان
کلروژنیک اسید
تشدید مغناطیسی هسته‌ای

چکیده

مقدمه: سرطان پدیده پیچیده‌ای است که عوامل طبیعی القا کننده آپوپتوز مانند ترکیب‌های فنولی می‌توانند در پیشگیری و درمان آن نقش موثری داشته باشند. از این رو اثرهای القا کنندگی آپوپتوز توسط میوه‌های گیاه زرشک که منبعی غنی از ترکیبات طبیعی فنولی هستند مورد توجه و مطالعه قرار گرفته است. **هدف**: این مطالعه با هدف ارزیابی اثرهای میوه گیاه زرشک بر القای آپوپتوز در سلول‌های سالم و سرطانی و میزان بیان ژن‌های p53، Bax و Bcl-2، بررسی مکانیسم احتمالی اثر ضد سرطان و شناسایی ترکیب موثر ضد سرطان صورت گرفت. **روش بررسی**: عصاره اتانولی میوه‌های گیاه زرشک به روش خیساندن تهیه گردید که با استفاده از روش کروماتوگرافی به فراکسیون‌های مختلف تقسیم و ساختار ترکیب موثر خالص شده بوسیله تشدید مناطیسی هسته‌ای تعیین گردید. همچنین تاثیر فراکسیون‌های منتخب بر علیه رده‌های سلولی 3T3 و MCF-7 و نیز mRNA ژن‌های تنظیم کننده آپوپتوز مانند p53، Bax و Bcl-2 متعاقبا بررسی گردید. **نتایج**: عصاره اتانولی میوه گیاه زرشک در سلول‌های سرطانی سطح mRNA ژن‌های p53 و Bax را افزایش و سطح mRNA ژن Bcl-2 را کاهش داد و ساختار ترکیب خالص شده نیز ۳ـکافوئیل‌کینیک‌اسید (کلروژنیک اسید) تشخیص داده شد. **نتیجه‌گیری**: میوه‌های گیاه زرشک در محیط برون تنی دارای اثرهای ضد نئوپلاسم هستند که با تحریک آپوپتوز سلولی بواسطه افزایش پروتئین‌های p53 و Bax و کاهش پروتئین Bcl-2 اعمال می‌شود. همچنین این میوه‌ها حاوی کلروژنیک اسید هستند که یک ترکیب طبیعی فنولی بوده و می‌تواند در اثرهای ضد سرطان مشاهده شده نقش داشته باشد.

مخففها: mRNA، ریبونوکلئیک اسید پیام رسان؛ HPLC، کروماتوگرافی مایع با کارآیی بالا؛ TLC، کروماتوگرافی لایه نازک؛ NMR، رزونانس مغناطیسی هسته‌ای؛ CC، کروماتوگرافی ستونی؛ MTT، ۳ـ(۴، ۵ـ دی متیل‌ـ۲ـ تیازولیل) ـ۲، ۵ـ دی فنیل ـ۲H‌ـ تترازولیوم بروماید؛ IC_{50}، نیمه حداکثر غلظت بازدارندگی؛ DMSO، دی متیل سولفوکساید؛ ELISA، سنجش جذب ایمنی وابسته به آنزیم؛ DOX، دوکسوروبیسین؛ PCR، واکنش زنجیره‌ای پلیمراز؛ cDNA، داکسی ریبو نوکلئیک اسید مکمل؛ ^{1}H-NMR، رزونانس مغناطیسی هسته‌ای پروتون؛ ^{13}C-NMR، رزونانس مغناطیسی هسته‌ای کربن ۱۳؛ BB، طیف گسترده؛ COSY، طیف سنجی همبسته؛ HMBC، هبستگی چندگانه ناجور هسته

* نویسنده مسؤول: sm.zarei@bmsu.ac.ir

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