J Kermanshah Univ Med Sci. 2022 December; 26(4):e131043. https://doi.org/10.5812/jkums-131043.

Published online 2023 January 19. **Research Article**

Evaluation of Molecular Docking and Cytotoxicity by *Pycnocycla bashagardiana* Fruit Essential Oil in Colon Cancer Cell Lines

Zahra Ghazazani [1], Masoumeh Heshmati [2,*], Jinous Asgarpanah [3] and Sanaz Pashapour [4]

[1] Pharmaceutical Sciences Center, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[2] Department of Cellular and Molecular Biology, Faculty of Advanced Sciences and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[3] Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[4] Department of Pharmacology and Toxicology, Faculty of Pharmacy and Pharmaceutical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

[*] *Corresponding author*: Department of Cellular and Molecular Biology, Faculty of Advanced Sciences and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran. Email: heshmati.cmb@gmail.com

Received 2022 August 26; **Revised** 2022 December 19; **Accepted** 2022 December 21.

Abstract

Background: *Pycnocycla bashagardiana* essential oil (EO) is extracted from fruits via hydrodistillation and analyzed through gas chromatography-mass spectrometry.
Objectives: This study aimed to evaluate molecular docking and cytotoxicity by *P. bashagardiana* EO in colon cancer cell lines.
Methods: The viability rates of *P. bashagardiana* fruit essential oil (PBFEO)-treated normal L929 and HT29 cancer cells were assessed at 0 - 500 μg/mL in 24 and 48 h by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The cell apoptosis percentage was investigated via flow cytometry and gene expression of BAX and BCL2 using the real-time polymerase chain reaction. In addition, the efficient myristicin binding was examined with the BCL2 protein through molecular docking analysis.
Results: The half maximal inhibitory concentrations of 431.8 and 450 μg/mL in L929 cells, as well as 278.5 and 144.6 μg/mL in HT29 cells, were recorded at 24 and 48 h, respectively. The cell apoptosis percentage was examined by annexin-V-FITC. The results indicated a significant increase in the apoptosis percentage. A significant increase was also observed in HT29 cancer cells compared to normal L929 cells regarding the expression ratio of BAX/BCL2 genes. Myristicin's binding affinity to BCL2 was relatively weak compared to the control.
Conclusions: Based on the results, myristicin can be suggested as a complementary treatment for colon cancer through more effective treatment and reducing the side effects of normal cells.

Keywords: *Pycnocycla bashagardiana*, Essential Oil, Cytotoxicity, Apoptosis

1. Background

Colon cancer is one of the most common and malignant cancers in men and women worldwide. Despite remarkable advances in diagnosing and treating colon cancer, the disease incidence is on the rise worldwide. Approximately 70 - 90% of colon cancers are related to lifestyle, physical activity, and dietary factors (1-4), and treatment strategies are available to fight cancer. On the other hand, there is no definitive cure for many types of cancer. Therefore, scientists decided to prioritize the move toward cancer prevention. The utilization of plants and their purified substances is prevalent, and many studied plants have been influential in treating cancer (2). This high potential of plants and their effective substances in treating and preventing cancer has led many scientists to study anti-cancer plants and seek to extract and identify their effective compounds worldwide, which are abundant in fruits, vegetables, grains, and cereals (5, 6). Many studies have shown the cytotoxic properties of oils, which increase treatment efficacy, reduce side effects, induce apoptosis, inhibit metastasis and angiogenesis in cancer cells, and decline drug resistance. Oils can be used alone or in combination with conventional cancer therapies (7, 8).

Pycnocycla belongs to the Apiaceae family, Apioideae subfamily, and Echinophora tribe. Pycnocycla includes 20 perennial, herbaceous, multicaulis, and spinous species in tropical and subtropical regions. About eight species of Pycnocycla, including *Pycnocycla bashagardiana*, are endemic to southern Iran and utilized in traditional medicine (9). The chemical composition analysis of the *P. bashagardiana* fruit essential oil (PBFEO) by Chehrazimeydan and Asgarpanah revealed the presence of myristicin (73.7%) as the major component (10). This finding characterized fruit oil as a rich natural source of myristicin as a phenylpropanoid

involved in several cellular processes such as cytotoxic activities (11, 12). There are limited studies on the cytotoxic effects of the *P. bashagardiana* fruit essence in colon cancer cell lines, and the standard treatment of colon cancer is surgery. In this regard, the Pycnocycla fruit essence can probably inhibit cancer cells and be effective.

2. Objectives

This research investigated the cytotoxic effects of the *P. bashagardiana* fruit essence in the colon cancer cell line using cell culture and flow cytometry methods.

3. Methods

3.1. Preparation of Pycnocycla bashagardiana

The *P. bashagardiana* fruits were collected from Bashagard Village, Jask County, Hormozgan Province, Iran (25°38'38" N, 57°46'28" E, 900 m) in September 2017. The ground-dried aerial parts of the plant were subjected to hydrodistillation in a Clevenger-type apparatus for 4 h. At the end of distillation, the ES was collected, dried with anhydrous Na_2SO_4, measured, transferred to a clean glass vial, and kept at an appropriate temperature until performing biological and analytical tests (9, 10).

3.2. Pycnocycla bashagardiana Analysis

The studied oil was analyzed using the gas chromatography-mass spectrometry technique to identify the potentially responsible compounds for the observed pharmacologic activities. The oil sample was examined using an HP-6890 GC device with an FID and a DB-5 capillary column. The quantitative data were collected through the electronic integration of the FID peak areas. The oil components were determined by the retention time and indicators of C9-C28 n-alkanes, computer matching with the WILEY275.L library, and comparison of the mass spectra of the components with those of original samples with previous works. The composition percentages of the detected compounds were estimated by the GC peaks areas without correction factors and computed relative to each other (9, 10).

3.3. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide Assay

About 6000 cells with Roswell Park Memorial Institute (RPMI) and 10% phosphate-buffered saline FBS medium were cultured in each well of a 96-well plate to perform the experiments. The cells were exposed to 12.5, 25, 50, 100, 250, and 500 μg/mL within 24 and 48 h after one day of incubation at 37°C. Control wells were selected as untreated with 20 mL of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) solution (5 mg/mL) added at the end of treatment. The optical density of samples was read by an enzyme-linked immunosorbent assay reader at 570 nm after 15 min of incubation at room temperature and complete dissolution of the crystals (13-15).

3.4. Apoptosis Assay

The apoptosis and necrosis percentages were analyzed using the Annexin-V-FLUOS pI kit. The cells were incubated in a 25 cm^2 plate in the RPMI medium. Then, concentrations lower than the half maximal inhibitory concentration (IC50) were selected from the results of the MTT method and added to the wells with three replications and incubated for 48 h. The cells were harvested with trypsin and collected after the treatment. Then, annexin-V-FLUOS labeled reagent solution, buffer, and propidium iodide (pI) solution (Sigma Aldrich, North America) were added to the target groups and incubated for 0.5h in the dark. Finally, 700 μL of the buffer was added to the samples and read by flow cytometry (FACSCalibur) and analyzed via FlowJO7.6.1 (2, 16).

3.5. Total RNA Extraction and Quantitative Real-time Polymerase Chain Reaction

The cells were cultured at 25000 to evaluate gene expression and treated at a defined concentration for 48 h after 24 h. Cellular RNA was extracted with the Yekta Tajhiz kit (Super RNA Extraction kit for Tissue & Cells, Cat: YT9080) based on the kit protocol. All real-time polymerase chain reactions (RT-PCRs) were performed in an ABI step-one system. The gene expression was evaluated using the SYBER Green quantitative polymerase chain reaction (qPCR) Master MIX (Amplicon) kit and performed at 95, 60, and 72°C for 15, 30, and 15 s, respectively. The gene expression analysis was evaluated with $2^{-\Delta\Delta Ct}$ (Livak) (Schmittgen Th, 2008). The gene primer sequences are shown in Table 1.

3.6. Molecular Docking Studies

A primary target in molecular docking was the susceptibility to evaluate the scoring function and estimate protein-ligand interactions to predict the activity and binding affinity of the ligand molecule. The BCL2 protein sequence was taken from the UniProt site, modeled by the Swiss model, and minimized by Swiss-pdbViewer software. The myristicin structure was downloaded in an SDF format from the PubChem site and converted to a PDB format by OPENABLE. The efficacy and binding of the myristicin ligand to the BCL2 protein were performed using AutoDock Vina software. ABT-199 was considered as a control and a

Table 1. Primer Sequences for Real-time Reverse-transcription Polymerase Chain Reaction Analysis

Gene and Primer Sequence	Product Size (bp)
BAX	169
5'TTGCTTCAGGGTTTCATCCAG3'	
5'AGCTTCTTGGTGGACGCATCC3'	
BCL2	170
5'TGTGGATGACTGAGTACCTGAACC3'	
5'CAGCCAGGAGAAATCAAACAGAG3'	
GAPDH	139
5'CCACTCCTCCACCTTTGACGCT3'	
5'TTACTCCTTGGAGGCCATGTGG3'	

clinical inhibitor of the BCL2 protein to compare the results of this study.

3.7. Statistical Analysis

GraphPad Prism software was used to analyze the results, draw charts, and compare the groups with one-way ANOVA and Tukey's post hoc test at a significance level of $P < 0.05$.

4. Results

4.1. Pycnocycla bashagardiana Analysis

The hydrodistillation of *P. bashagardiana* fruits generated a yellowish oil with a strong odor and 0.8% (v/w) yield based on the fresh weight. Figure 1 shows the gas chromatogram of PBFEO analysis. Based on Table 2, 12 compounds were specified in the oil, accounting for almost 98.7% of the total chromatographical material, and myristicin (75.1%) was the main oil constituent.

4.2. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide Measurement

Figure 2 shows the viability rates of PBFEO-treated normal L929 and HT29 cancer cells decreased at 0 - 500 μg/mL in 24 and 48 h. The viability decrease in normal L929 cells was significant only at 500 μg/mL compared with the control group ($P \leq 0.0001$), and the highest (99.733 and 85.729%) and lowest (41.422 and 44.713%) viability rates were shown at 12.5 and 500 μg/mL in 24 and 48 h, respectively. The IC50 concentrations of 431.8 and 450 μg/mL were recorded in 24 and 48 h, respectively. The highest (99.9 and 90.65%) and lowest (38.371 and 21.068%) viability rates in HT29 cancer cells were found at 12.5 and 500 μg/mL in 24 and 48 h, respectively. The IC50 concentrations of 278.5 and 144.6 μg/mL were detected in 24 and 48 h, respectively (15, 17).

Table 2. Gas Chromatography-mass Spectrometry Analysis of *Pycnocycla bashagardiana* Fruit Essential Oil

Compound [a]	KI [b]	KI [c]	Percentage
1. Sabinene	972	975	1.3
2. β-Pinene	976	979	7.9
3. Z-β-Ocimene	1035	1037	1.4
4. E-β-Ocimene	1052	1050	3.8
5. β-Cubebene	1394	1391	1.6
6. Methyl eugenol	1396	1401	0.9
7. α-Guaiene	1445	1440	1.2
8. δ-Guaiene	1505	1508	1.1
9. Myristicin	1523	1520	75.1
10. Caryophyllene oxide	1588	1583	0.4
11. Isomyristicin	1627	1624	1.5
12. β-Eudesmol	1655	1651	2.5
Total			98.7

[a] Compounds listed in the order of elution
[b] KI: Kovats index measured relative to n-alkanes (C_9 - C_{28}) on the non-polar DB-5 column under conditions listed in the Materials and Methods Section
[c] KI: Kovats index from the literature

4.3. Cell Apoptosis

Based on the MTT test results, the apoptosis percentages of L929 and HT29 cancer cell lines treated with PBFEO were tested at three concentrations of 50, 100, and 250 μg/mL in 48 h. An increase in apoptosis was observed in normal L929 cells treated with PBFEO at 50 (2.9 fold), 100 (3.8 fold), and 250 μg/mL (2.1 fold) compared with untreated cells. Further, a significant difference was detected only at 100 μg/mL compared with untreated cells ($P < 0.05$). A significant increase was shown in necrosis at 250 μg/mL ($P < 0.001$). Apoptosis in HT29 cancer cells treated with PBFEO at 50 and 100 g/mL increased 5.08 and 10.4 times with a significant difference from untreated cells. Furthermore, significant necrosis was detected compared with the untreated cells at 250 μg/mL ($P < 0.0001$). The apoptosis rates resulting from treating HT29 cancer cells and normal L929 cells at 50 and 100 μg/mL of PBFEO were compared by statistical analyses. Significant (3.2% ($P < 0.001$) and 5.1 ($P < 0.0001$)) increases were observed in the apoptosis rate in HT29 cancer cells compared with L929 cells at 50 and 100 μg/mL of PBFEO, respectively.

4.4. The Expression of BCL2 and BAX Genes

As shown in Figure 3, there are a significant decrease in normal L929 cells treated with PBFEO in fold changes and relative expression of the *BCL2* gene at 50 ($P < 0.0001$) and 100 μg/mL ($P < 0.01$), respectively. The fold changes

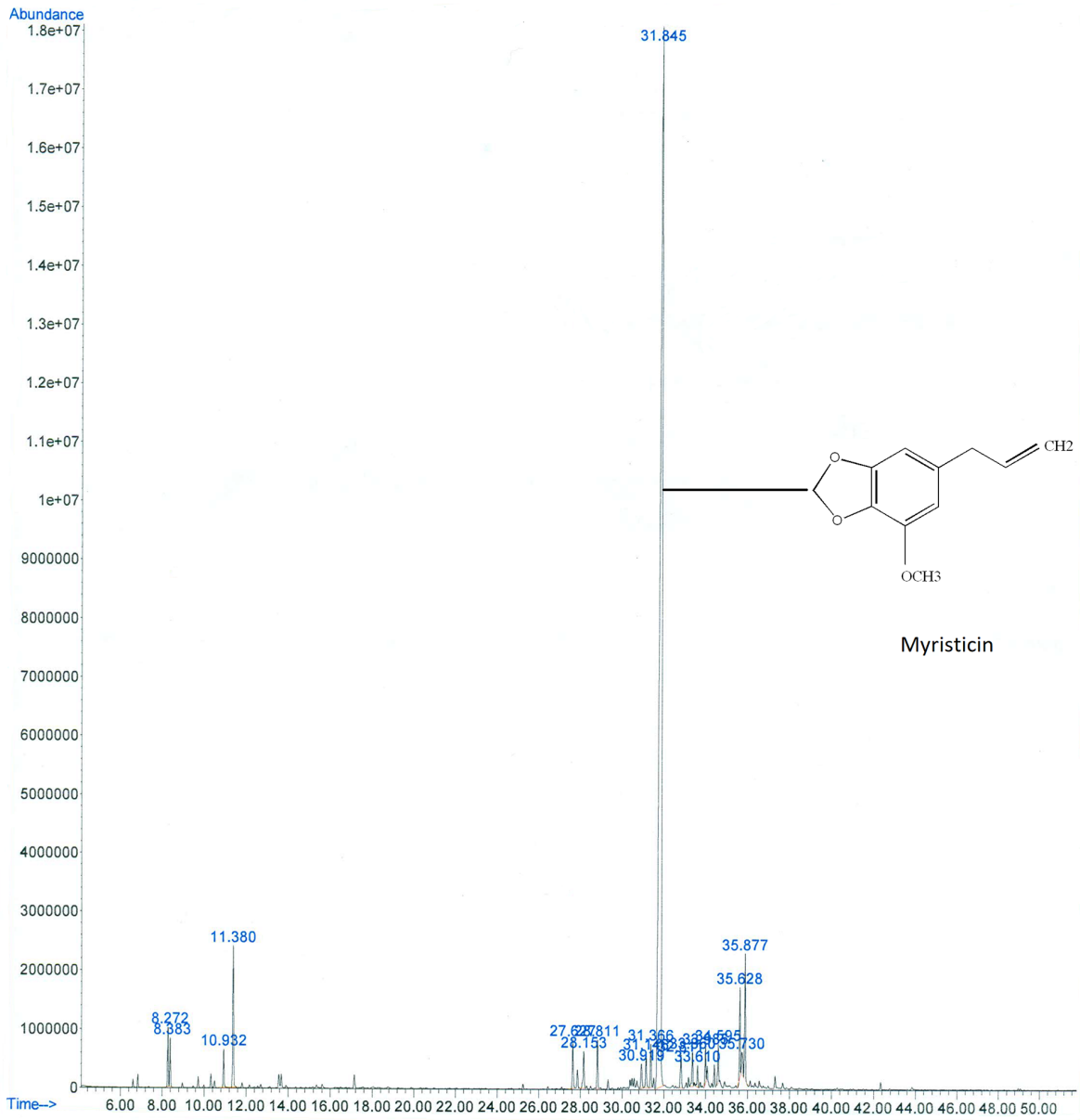

Figure 1. Gas chromatogram of *Pycnocycla bashagardiana* fruit essential oil analysis

in the relative expression of the *BAX* gene significantly decreased at 50 and 100 μg/mL compared to the control ($P < 0.0001$). A significant decrease occurred in the fold change at 50 μg/mL ($P < 0.01$), which non-significantly reduced the relative expression of the *BCL2* gene in PBFEO-treated HT29 cancer cells at 100 μg/mL. On the other hand, significant increases were found in the relative expression of the *BAX* gene at 50 and 100 μg/mL ($P < 0.0001$) compared with untreated cells. Based on Figure 3, the BAX/BCL2 ratios decrease in the normal L929 cell treated with PBFEO at 50 and 100 μg/mL compared to the HT29 colon cancer cell line.

4.5. Protein-Ligand Docking

Figure 4A depicts the BCL2 protein, and Figure 4B indicates the efficacy and binding of the myristicin ligand to the BCL2 protein. H-bonding interactions between the myristicin ligand and BCL2 protein target are illustrated in Figure 4C. Val93, Leu97, Asp103, Phe104, Arg106, and Arg107

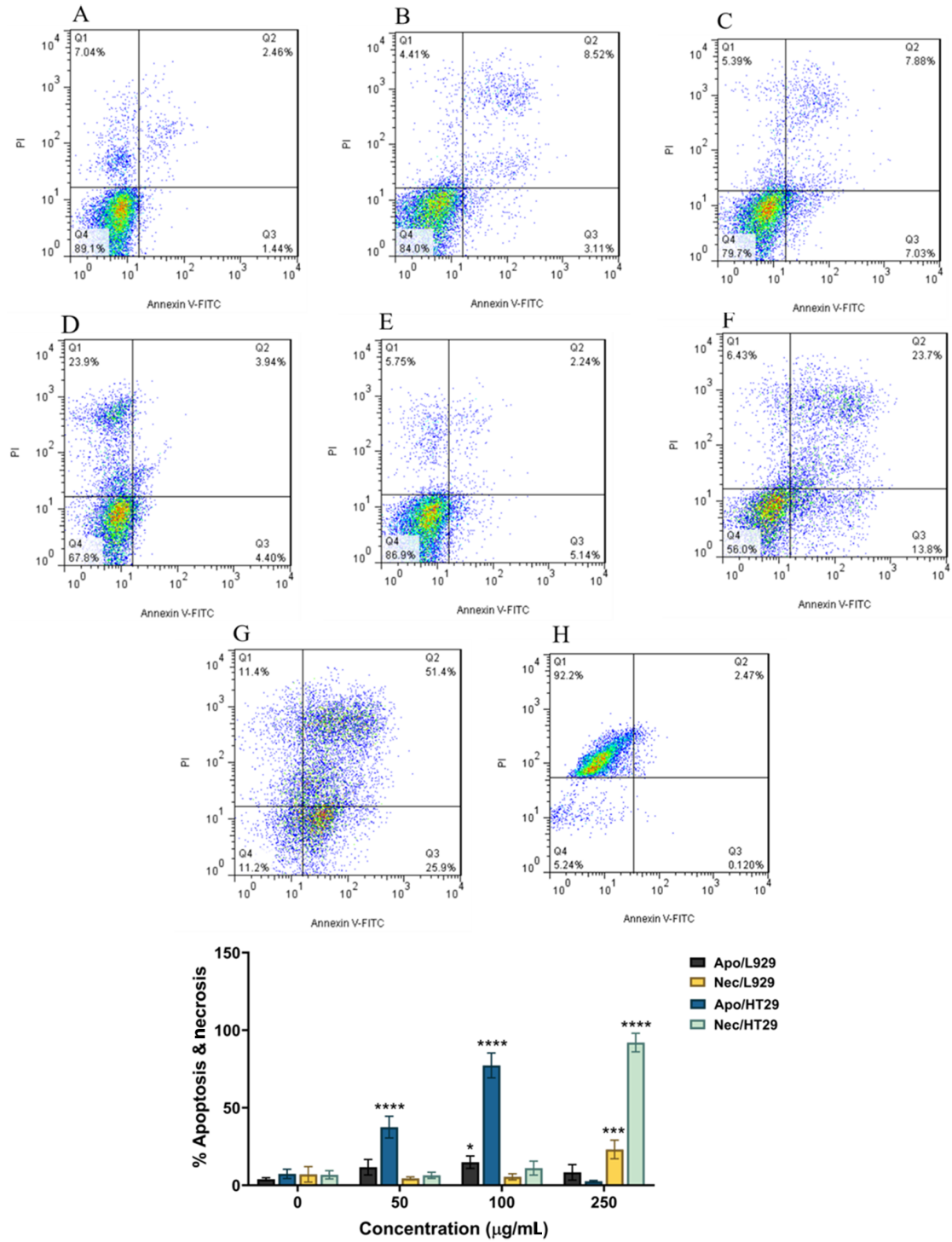

Figure 2. Comparison of the effects of *Pycnocycla bashagardiana* fruit essential oil (PBFEO) concentrations on the viability of L929 and HT29 cell lines at 24 and 48 h. The results are presented as the means ± standard deviation (SD). * $P < 0.05$, * $P < 0.01$, *** $P < 0.001$, **** $P < 0.001$ compared with the control group

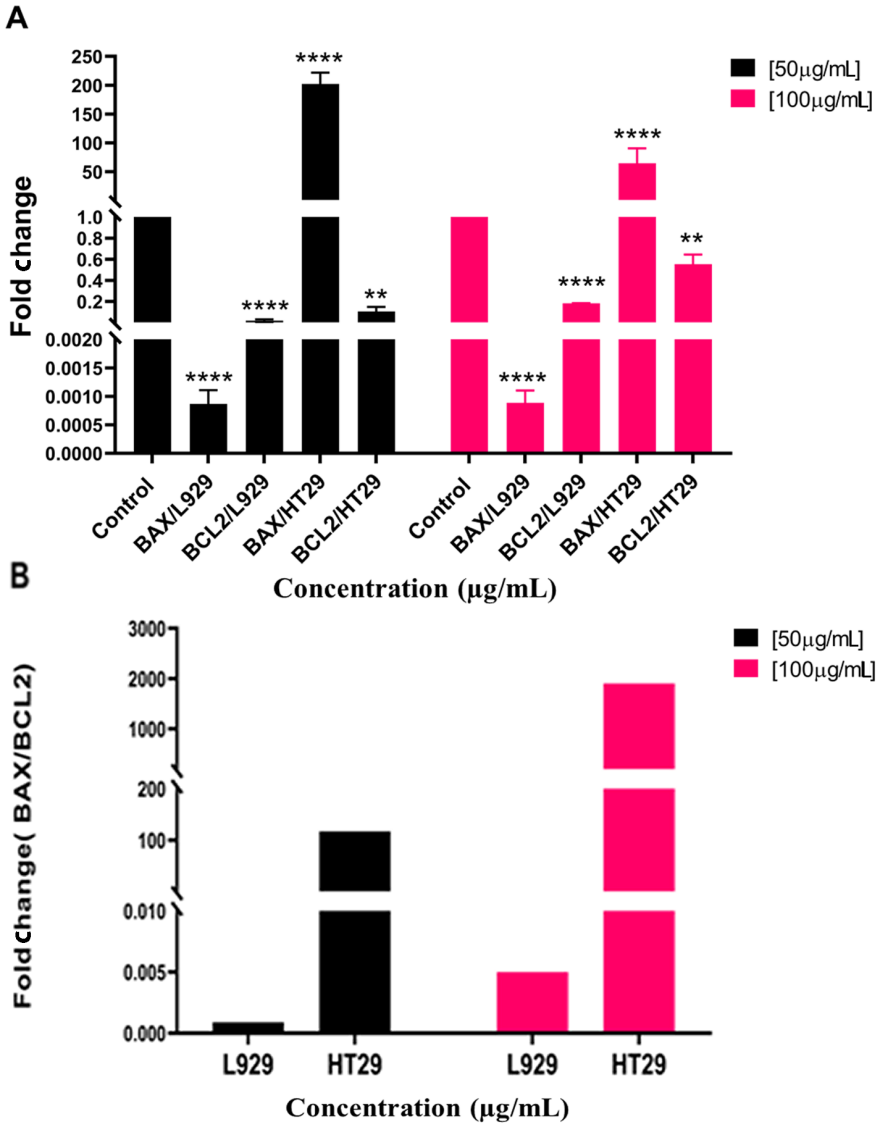

Figure 3. Changes in the expression of BAX and BCL2 genes in the L929 and HT29 cell lines treated with *Pycnocycla bashagardiana* fruit essential oil (PBFEO) at 50 and 100 μg/mL for 48 h; A, * Denotes significance relative to the control group, ** $P < 0.01$ and **** $P < 0.0001$ compared with the control group; B, BAX/BCL2 gene expression ratio in L929 and HT29 cell lines treated with PBFEO at 50 and 100 μg/mL for 48 h.

amino acids were considered as the binding sites for the BCL2 protein. The binding energy of the myristicin ligand to the BCL2 protein is numerically equal to -5.3 (energy binding for ABT-199 equals -7.5). Van der Waals forces were observed in the A: GLY145: H and A: ASN143: OD1 region of the BCL2 protein. As a result, the ligand binding to the protein can occur in this region. Although myristicin has a weaker binding affinity than the control, it is still acceptable.

5. Discussion

The effect of PBFEO fruit essence on reducing the proliferation of colon cancer cells remains a controversial topic despite the ability of the PBFEO plant to inhibit cancer cells (11, 12). Accordingly, the current study used MTT assay, flow cytometry, and RT-PCR methods to investigate the cytotoxic effects of the PBFEO fruit essence. The study demonstrated that PBFEO fruit essence could destroy colon cancer cells through cellular and molecular mechanisms. In this regard, plants rich in metabolites such as alkaloids,

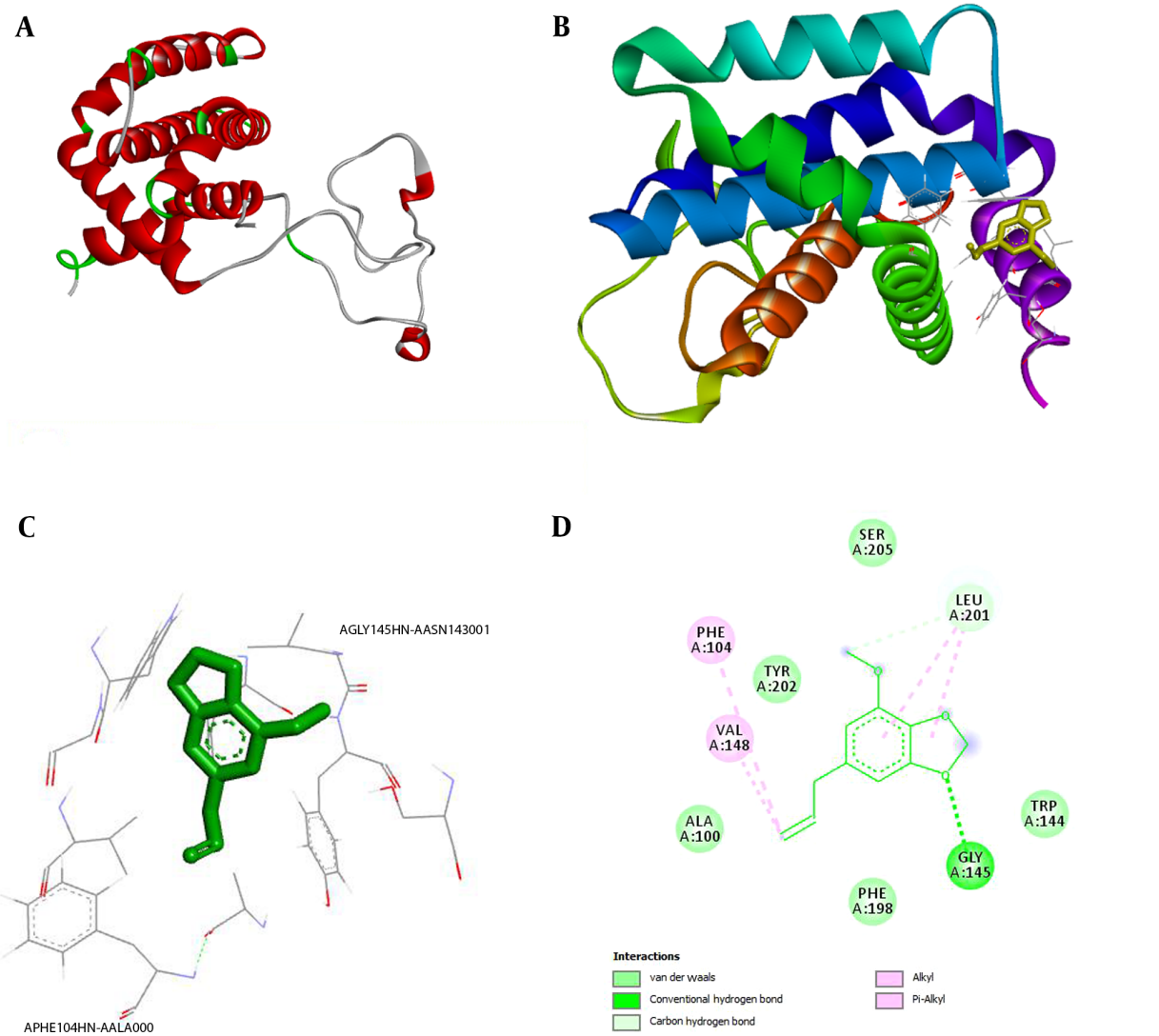

Figure 4. A, Modeling of BCL2 protein; B, Binding of Myristicin to BCL2; C, H-bonding interactions between the myristicin ligand and BCL2 protein target; and D, All types of interactions between the ligand and protein.

flavonoids, and terpenoids were focused on their historical records in cancer treatment (5, 18-20). The results demonstrated that myristicin is the main component of the oil (75.1%). Several studies have explored myristicin and myristicin-containing herbal extracts' anti-inflammatory, anti-cancer, and apoptotic effects in animal models and cancer cell lines. The mitochondria pathway plays a role in causing apoptosis by activating caspase enzymes. The BCL2 protein family is a regulator of mitochondria, which is one of the essential anti-apoptotic proteins in living cells. BAX is considered as an inactive protein in the cytosol or membrane as a monomer. The increased BAX expression compared to BCL2 activated the caspase cascade and inducing apoptosis in apoptotic cells (19, 20). *Pycnocycla bashagardiana* fruit essential oil was evaluated on the HT29 cancer cell line and the L929 cancer cell line for its antiproliferative effect and apoptosis induction. In agreement with these findings, a study reported that the EO prepared from *Myristica fragrans* contained 32.8% myristicin, which inhibited the growth of $CaCO_2$ cancer cells (18). Decreased cell viability was observed in cancerous and normal cells after treatment with different concentrations of PBFEO.

Moreover, the inhibition of cell proliferation is concentration-dependent. A significant increase in

apoptosis was found in HT29 cancer cells compared to the control (untreated). The effects of the mace extract from *M. fragrans* against *Helicobacter pylori* as an anti-inflammatory property were reported on the RAW264.7 cell line. Additionally, antioxidant and anti-cancer effects were found on the KATO III gastric cancer cell line (20). The researchers have observed decreased cell viability, accumulated cytochrome c, activated CASPASE 3, and induced apoptosis (21). The carcinoma KB cell line treated with the *M. fragrans* mace extract could inhibit cancer cells and reduce the expression of the *BCL2* gene (22). Another study confirmed the anti-violence effect of *P. bagardiana* EO on male Wistar rats (23). In this study, the gene expression results revealed a significant increase in the relative expression ratio of BAX/BCL2 genes in cancer cells compared to normal cells. The rise in *BAX/BCL2* gene ratio had a crucial role in the mitochondria in PBFEO-induced apoptosis. Zhang et al. as cited in Zengin et al. demonstrated the antimicrobial effects of *P. peucedanifolia* and *P. ferulacea* plants on *Staphylococcus aureus* and *Bacillus cereus* as Gram-positive bacterial strains. The antioxidant and cytotoxic effects were reported on the HepG2 hepatocarcinoma cell line and murine bone marrow stromal cell line S17 (24). According to previous evidence, EOs did not have a specific cellular target, crossing the cell membrane and causing damage to the membrane due to their lipophilic properties. Essential oils (EOs) can be used as an antitumor in treating cancers due to their peroxidation property, mitochondrial function intervention, free radical production, and lack of side effects on healthy tissues (25).

A study confirmed the cytotoxic and genotoxic effects of myristicin and 1'-hydroxymyristicin after exposure to HepG2 cells for 24 h (26). Another study investigated the cytotoxic and apoptotic effects of myristicin on SK-N-SH human neuroblastoma cells and reported that this compound could cause apoptosis in this cell line by cytotoxicity (21). In the present study, myristicin ligand binding to the BCL2 protein was analyzed using molecular docking analysis. The weak binding affinity of myristicin to BCL2 was reported compared to the control, which can be considered a factor in inducing fewer side effects in healthy cells. Therefore, diets with EOs rich in myristicin were considered as a cancer treatment.

Further extensive in vitro and in vivo studies are needed to better investigate the anti-cancer effects of PBFEO. However, more extensive research is necessary to accurately analyze the effects of the PBFEO fruit EO on colon cancer cells. This study evaluated the cytotoxic effects of the PBFEO fruit EO on colon cancer cells and fibroblast cells in the cell culture. This research could clarify the relationship between the PBFEO fruit EO and colon cancer to manage and prevent liver cancer.

5.1. Conclusions

The results showed the decreased viability rates of normal L929 and HT29 cancer cells treated with different PBFEO concentrations. On the other hand, HT29 cancer cells showed an increase in apoptosis and BAX/BCL2 gene expression compared with normal L929 cells. The cytotoxic and apoptotic effects were observed by treating HT29 cancer cells with PBFEO and the weak binding efficiency of myristicin to the BCL2 protein, which can be suggested as a complementary treatment in colon cancer through more effective treatment and a decline in the side effects of normal cells.

Acknowledgments

This study was financially supported by the Islamic Azad University of Pharmaceutical Sciences Branch.

Footnotes

Authors' Contribution: Study concept: Z. Gh. and M. H.; analysis and interpretation of data: J. A.; drafting of the manuscript: S. P.; critical revision of the manuscript for important intellectual content: Z. Gh.; statistical analysis: M. H.

Conflict of Interests: The authors declared no conflict of interests.

Data Reproducibility: The dataset presented in the study is available upon request from the corresponding author during submission or after publication.

Funding/Support: There is no funding support.

References

1. Pashapour S, Heshmati M, Mousavi Z, Esmaeili S. The Cytotoxicity of the Chloroform and Petroleum Ether Fractional Extracts of Galium verum L. in HepG2 and HT29 Cell Lines. *J Kermanshah Univ Med Sci*. 2020;**24**(2). https://doi.org/10.5812/jkums.101079.
2. Zabihi A, Pashapour S. Therapeutic effects of the Galium verum. *Food Therapy and Health Care*. 2022;**4**(3). https://doi.org/10.53388/fthc20220801014.
3. Wdowiak A, Farahmandlou N, Tajik A, Pashapour S, Ahmadi R. The Cytotoxic Effect of Estradiol Valerate, Progesterone, and Testosterone on Brain Glioblastoma (A172), Colorectal Cancer (HT29) and Human Embryonic Kidney (HEK293) Cells and the Expression Levels of Bax, Bcl-2, and KAI-1/CD82 in A172 and HT29 cells. *Journal of Biological Studies*. 2021;**4**(3):106–19.
4. Pashapour S, Heshmati M, Mousavi Z, Esmaeili S. The apoptotic effect of methanolic extract of Galium verum on HT29 cell line. *Journal of Biological Studies*. 2022;**4**(4):210–20.
5. Pashapour Z, Heshmati M, Mousavi Z, Esmaeili S. Effect of whole methanolic extract of Galium verum on AGO cell line. *Toxicol Commun*. 2022;**4**(2):10.

6. Perricone M, Arace E, Corbo MR, Sinigaglia M, Bevilacqua A. Bioactivity of essential oils: a review on their interaction with food components. *Front Microbiol*. 2015;**6**:76. [PubMed ID: 25709605]. [PubMed Central ID: PMC4321600]. https://doi.org/10.3389/fmicb.2015.00076.
7. Russo R, Corasaniti MT, Bagetta G, Morrone LA. Exploitation of cytotoxicity of some essential oils for translation in cancer therapy. *Evid Based Complement Alternat Med*. 2015;**2015**:397821. [PubMed ID: 25722735]. [PubMed Central ID: PMC4334976]. https://doi.org/10.1155/2015/397821.
8. Christensen LP, Brandt K. Bioactive polyacetylenes in food plants of the Apiaceae family: occurrence, bioactivity and analysis. *J Pharm Biomed Anal*. 2006;**41**(3):683–93. [PubMed ID: 16520011]. https://doi.org/10.1016/j.jpba.2006.01.057.
9. Hosseaini RS, Asgarpanah J, Bakhtiarian A, Mousavi Z. Phytochemical Analysis, Antinociceptive and Anti-inflammatory Effects of Pycnocycla bashagardiana Aerial Parts Essential Oil in Experimental Animals. *Journal of Medicinal plants and By-product*. 2021.
10. Chehrazimeydan M, Asgarpanah J. Essential Oil Chemical Compositions of the Fruit and Seeds of the Endemic Species Pycnocycla Bashagardiana Mozaff. *J Chil Chem Soc*. 2015;**60**(3):3038–9. https://doi.org/10.4067/s0717-97072015000300013.
11. Seneme EF, Dos Santos DC, Silva EMR, Franco YEM, Longato GB. Pharmacological and Therapeutic Potential of Myristicin: A Literature Review. *Molecules*. 2021;**26**(19). [PubMed ID: 34641457]. [PubMed Central ID: PMC8512857]. https://doi.org/10.3390/molecules26195914.
12. Budisan L, Gulei D, Zanoaga OM, Irimie AI, Sergiu C, Braicu C, et al. Dietary Intervention by Phytochemicals and Their Role in Modulating Coding and Non-Coding Genes in Cancer. *Int J Mol Sci*. 2017;**18**(6). [PubMed ID: 28587155]. [PubMed Central ID: PMC5486001]. https://doi.org/10.3390/ijms18061178.
13. Norouzi S, Ahmadi R, Pashapour S. [The cytotoxic effects of Tolmetin on evaluation of Bax and Bcl2 genes expression level in cervical cancer cells (Hela)]. *Feyz*. 2020;**24**(1):31–7. Persian.
14. Zabihi A, Pashapour S, Malakijoo N. Evaluation of cytotoxicity of rubiadine on MCf7 and AGO cell lines. *Toxicol Commun*. 2022;**4**(2). https://doi.org/10.53388/2022020209.
15. Heshmati M, Hasani-Reza Abad N, Pashapour S. Evaluating the Effects of Silymarin on Expressing SBDSP1 and CASC11 Genes in HCT116 Colon Cancer Cells. *J Kermanshah Univ Med Sci*. 2022;**26**(2). https://doi.org/10.5812/jkums-122802.
16. Pashapour S, Heshmati M, Mousavi Z, Esmaeili S. The effects of methanolic extract of the aerial parts of Galium verum on HT29 and AGO cell lines. *Nucleus*. 2021;**65**(2):223–32. https://doi.org/10.1007/s13237-021-00380-1.
17. Pashapour S, Zabihi A, Behrouzi R. Investigating the cytotoxic effect of ibuprofen concentration in liver cancer cells (HepG2) and normal fibroblast (AGO). *Toxicol Adv*. 2022;**4**(4). https://doi.org/10.53388/20220202015.
18. Piras A, Rosa A, Marongiu B, Atzeri A, Dessi MA, Falconieri D, et al. Extraction and separation of volatile and fixed oils from seeds of Myristica fragrans by supercritical CO(2): chemical composition and cytotoxic activity on Caco-2 cancer cells. *J Food Sci*. 2012;**77**(4):C448–53. [PubMed ID: 22429024]. https://doi.org/10.1111/j.1750-3841.2012.02618.x.
19. Martins C, Doran C, Silva IC, Miranda C, Rueff J, Rodrigues AS. Myristicin from nutmeg induces apoptosis via the mitochondrial pathway and down regulates genes of the DNA damage response pathways in human leukaemia K562 cells. *Chem Biol Interact*. 2014;**218**:1–9. [PubMed ID: 24792648]. https://doi.org/10.1016/j.cbi.2014.04.014.
20. Suthisamphat N, Dechayont B, Phuaklee P, Prajuabjinda O, Vilaichone RK, Itharat A, et al. Anti-Helicobacter pylori, Anti-Inflammatory, Cytotoxic, and Antioxidant Activities of Mace Extracts from Myristica fragrans. *Evid Based Complement Alternat Med*. 2020;**2020**:7576818. [PubMed ID: 32308716]. [PubMed Central ID: PMC7146089]. https://doi.org/10.1155/2020/7576818.
21. Lee BK, Kim JH, Jung JW, Choi JW, Han ES, Lee SH, et al. Myristicin-induced neurotoxicity in human neuroblastoma SK-N-SH cells. *Toxicol Lett*. 2005;**157**(1):49–56. [PubMed ID: 15795093]. https://doi.org/10.1016/j.toxlet.2005.01.012.
22. Rengasamy G, Venkataraman A, Veeraraghavan VP, Jainu M. Cytotoxic and apoptotic potential of Myristica fragrans Houtt. (mace) extract on human oral epidermal carcinoma KB cell lines. *Braz J Pharm Sci*. 2018;**54**(3). https://doi.org/10.1590/s2175-97902018000318028.
23. Jahandar F, Asgarpanah J, Najafizadeh P, Mousavi Z. Anti-inflammatory activity and chemical composition of Pycnocycla bashagardiana fruit's essential oil in animal models. *Iran J Basic Med Sci*. 2018;**21**(2):188–93. [PubMed ID: 29456816]. [PubMed Central ID: PMC5811758]. https://doi.org/10.22038/IJBMS.2017.20860.5426.
24. Zengin G, Sinan KI, Ak G, Mahomoodally MF, Paksoy MY, Picot-Allain C, et al. Chemical profile, antioxidant, antimicrobial, enzyme inhibitory, and cytotoxicity of seven Apiaceae species from Turkey: A comparative study. *Ind Crops Prod*. 2020;**153**. https://doi.org/10.1016/j.indcrop.2020.112572.
25. Kalmar A, Nagy ZB, Galamb O, Csabai I, Bodor A, Wichmann B, et al. Genome-wide expression profiling in colorectal cancer focusing on lncRNAs in the adenoma-carcinoma transition. *BMC Cancer*. 2019;**19**(1):1059. [PubMed ID: 31694571]. [PubMed Central ID: PMC6836529]. https://doi.org/10.1186/s12885-019-6180-5.
26. Marabini L, Neglia L, Monguzzi E, Galli CL, Marinovich M. Assessment of Toxicity of Myristicin and 1'-Hydroxymyristicin in HepG2 Cell Line. *J Pharmacol Toxicol*. 2017;**12**(4):170–9. https://doi.org/10.3923/jpt.2017.170.179.