



Effect of *Adiantum capillus-veneris* Extract on Expression of P16 and *Bax* genes in breast cancer (MCF-7) cell line

Mayyadah Fakhir Jaafer, Wafaa Sabri Mahood

Department of Biology, College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.

Article Info

Article history:

Received 03, 06, 2025

Revised 04, 10, 2025

Accepted 05, 20, 2025

Published 10, 30, 2026

Keywords:

Adiantum capillus-veneris L.

P16 gene

Bax gene

MCF-7

Breast cancer

ABSTRACT

Medicinal herbs are intriguing and have expanded as potential therapeutic agents against cancer cells. Identification of plant extracts is the most important field of research worldwide. This study aimed to investigate the possible growth-inhibitory effect of the aerial part of *Adiantum capillus-veneris* L. (ACV) extract on the MCF-7 breast cell line, in addition to detecting the *p16* and *Bax* gene expression levels, as they play an important role in the cell cycle and initiate breast cancer. The extract of *Adiantum capillus-veneris* L. was prepared using an ethanol solution. The toxicity of the ACV extract was measured by MTT assay with different drug concentrations (1000, 500, 250, 125, and 62.5 µg/mL) for 24 hrs. Detection of *P16* and *Bax* mRNA expression by real-time PCR. The MTT assay results showed that 1746 µ/mL of ACV extract had 50% toxicity on MCF-7 cells after 24 hours of incubation. Real-time PCR results showed an increased expression of *P16* *Bax*. In conclusion, ACV extract significantly overexpressed P16 and BAX proteins in MCF-7 cells, suggesting therapeutic potential for breast cancer.

This is an open access article under the [CC BY](#) license.



Corresponding Author:

Wafaa Sabri Mahood

Department of Biology, College of Education for Pure Science, Ibn-AlHaitham

University of Baghdad,

Baghdad city, Baghdad Governorate, Iraq.

Email: wafaa.s.m@ihcoedu.uobaghdad.edu.iq



1. INTRODUCTION

Cancer is considered the most important invasive cancer in the world, accounting for thirty percent of cancers in females, according to the World Health Organization [1]. Women with specific genetic variations are predisposed to a higher risk of breast cancer [2]. Cell proliferation is a critical process regulated by inhibition of the cell cycle and apoptosis (programmed death). Various suppressor genes for both cell cycle arrest and programmed death include tumor suppressor genes such as p53, one of the most important mutated genes in human cancers; it controls cell proliferation in response to DNA damage. Other tumor suppressor genes, including P27Kip1, P21Waf1, and P16INK4A, also regulate the cell cycle by inhibiting the collision of cyclins with cyclin-dependent kinases [3].

P16 is a tumor suppressor protein that is encoded by the P16INK4a gene located in 9p21 [4]. P16 acts as an inhibitor of cyclin-dependent kinases (CDKs). It slows down the cell cycle by forbidding progression from the G1 phase to S [5]; in the absence of P16 protein, CDK4/6 binds and forms an active protein complex that induces the phosphorylation of retinoblastoma protein (pRB) [6], causing dissociation of pRB from the transcription factor E2F1. The liberation of E2F1 allows it to be transported from the cytoplasm to the nucleus, where it promotes the transcription of necessary genes for transition from G1 to S phase in the cell cycle [7]. With telomerase activation, cells may certainly progress to proliferation, immortality, and cancer [8]. Two molecular pathways induce apoptosis: one mediated by Fas and tumor necrosis factor, called an external pathway, and the other focused on the release of cytochrome C from the mitochondria, called an intracellular pathway [9].

The Bcl-2 apoptosis-related, p53-dependent protein family is one of the main regulators of the second pathway and includes both apoptosis-activating members, such as Bak and Bax, and inhibitors, such as Bcl-2 and Bcl-x. In breast cancer, the *Bax* gene plays an important role as an activator of apoptosis; its function is

necessary for balance among the main antiapoptotic members of the family, Bcl-2 [10, 11]. The extracts of various medicinal plants have been used as antibacterial and antifungal agents [12-16], with an anti-diabetic effect [17]. Several studies have shown that plant extracts and their essential oils have an anticancer effect against several human cancer cell lines [18-21].

Adiantum capillus-veneris is one of the Pteridaceae family. About 200 species of ferns belong to this family. They are widely spread throughout the world, from hot tropical regions to cold temperate zones [22]. The medicinal portions of *Adiantum capillus-veneris* include fronds, rhizomes, and roots [23]. Among its various secondary metabolites, *Adiantum capillus-veneris* shows antibacterial, anti-inflammatory, analgesic, hypoglycemic, antioxidant, antilithiasic, antiproliferative, antidermatitis, neuroprotective, and anticholesterolemic properties [24].

The current study was designed to assess the cytotoxic activity of *Adiantum capillus-veneris* L. in MCF-7 breast cancer cells and address the gene expression of the *P16* and *Bax* genes as candidate genes for cancer cell proliferation.

2. METHOD

2.1. This study included the use areal parts of *Adiantum capillus-veneris* L. The plant was obtained from local markets in Baghdad. The plant was identified in herbarium, Department of Biology College of Science - University of Baghdad. The plant aerial parts grinded in to powder form by mechanical grinder.

2.2. Ethanol extraction preparation

Ethanol (Alcoholic) plant extract were prepared according to [25] with some modifications. The dried powder of (*Adiantum capillus-veneris* L.) plant (40 g) were placed separately in the thimble of Soxhlet apparatus. The extraction solvent used was 400 ml of 95% ethanol. The solvent was heated to reflux at 100°C for 8 h. After the extraction, the products were collected and concentrated using rotary evaporator at 50°C, then left under fume hood for one hour to make sure all the ethanol left in the extract crude was completely vaporized to the environment. The solution was input into oven to derided, and were kept in refrigerator in tight containers for experiments.

2.3. Cell culture

The MCF-7 cell line was purchased from Cell Technologies Lab—Iraq. The cells were cultured in MEM (US Biological, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Capricorn Scientific, Germany) and 100 IU penicillin, and 100 µg streptomycin (Capricorn Scientific, Germany) and incubated in a humidified atmosphere at 37°C. Exponentially growing cells were used for experiments [26].

2.4. Cytotoxicity Assay

An in vitro experiment was performed using 96-well plates. 10000 cells were plated in a 96-well microplate (NEST Biotech, China) and cultured for seventy-two hours at 37°C until monolayer confluence was reached. The growth medium was removed, and the sticky cells were cleaned with Phosphate Buffered Saline (PBS) once the cells had achieved 80% confluence. After adding 1 mL of trypsin-EDTA solution, the flask was gently shaken to thoroughly cover the monolayer. A cell proliferation experiment based on MTT was used to evaluate the cytotoxicity potential of ACV extract.

Human breast cancer cells (MCF-7) were cultured for 24 hours at 37°C in a humidified environment with 5% CO₂ after being exposed to several doses of ACV extract (1000, 500, 250, 125, 62.5, 31.2 µg/mL, and no treatment as a negative control). Each concentration was tested in three replicates. After exposure, each well received 28 µL of MTT stain solution (2 mg/mL). Plates were then incubated at 37°C with 5% CO₂ for 3 hours. After carefully removing the medium, 100 µL of DMSO was added to each well and incubated for 15 minutes to dissolve the MTT-formazan crystals in accordance with [27]. Then the plate was ready for reading on the ELISA reader at 492 nm. The inhibitory concentration rate was calculated as a drug concentration as follows [28]:

$$\text{Cytotoxicity \%} = (\text{OD Control} - \text{OD sample}) / \text{OD Control} \times 100$$

Where OD control is the mean optical density of untreated wells, and OD Sample is the optical density of treated wells. Experimental data on variance were utilized for data comparison between study groups (control and samples) using 3 replicates for each experiment. A Student's t-test was used to determine the results. A P value < 0.05 indicated statistical significance. We performed this research using GraphPad Prism 8 software (GraphPad Software, Inc., San Diego, California). The 50% growth inhibition concentration (IC₅₀) was calculated from a plotted dose-response curve. The IC₅₀ value is represented by the log of the ACF concentration that inhibits 50% of cell viability.

2.5. Detection of gene Expression

Gene expression was detected by quantitative real-time polymerase chain reaction (qRT-PCR) technique. Table 1 refers to the primer sequences used in this study. Total RNA was extracted from untreated (negative control) and treated MCF-7 cells at an IC₅₀ of 1746 µg/m for 24 hrs. This was performed using a kit (GoTaq® 1-Step RT-qPCR System) and TRIzol Reagent (Thermo Scientific, USA). This study depends on a one-step qRT-PCR procedure (Mic qPCR Cycler, Bio Molecular System, Australia) in a 10 µl reaction volume containing 5 µl qPCR Master Mix and 0.25 x RT mix. 0.25 MgCl₂ and 0.3 µM for each (forward and reverse primer, 2.5 µl nuclease-free water, and 1 µl of RNA. Detection of P16 and Bax gene expression levels was done by RT-qPCR technique using the GEGF gene as a housekeeping gene in this study. Table 2 indicates that for the PCR program, all primers were purchased from Macrogen, Korea. The results of gene expression were analyzed using relative quantification, which measures the relative change in mRNA expression levels (Pfaffl method) (Folding = 2- $\Delta\Delta CT$, $\Delta\Delta CT = \Delta CT$ Treated - ΔCT Control, $\Delta CT = CT$ gene - CT Housekeeping gene) [29].

Table 1. Primers sequences for quantitative real-time-

Gene Polymorphism Primers			Reference.
Primer Name	Sequence (5' → 3')	Annealing Temp. (°C)	
TEGT-F	TGCTGGATTTGCATTCCTTACA	60	
TEGT-R	ACGGCGCCTGGCATAGA		
p16-F	AGCCTTCGGCTGACTGGCTGG	60	[30]
p16-R	CTGCCCAT CATCATGACCTGGA		
BAX -F	CCAAGAAGCTGAGCGAGTGT	54.5	[31]
BAX -R	CCGGAGGAAGTCCAATGTC		

Table 2. Real Time PCR Program

Steps	°C	m: s	Cycle
RT. Enzyme Activation	37	15:00	1
Initial Denaturation	95	05:00	
Denaturation	95	00:20	40
Annealing	54.5 or 60	00:20	
Extension	72	00:2	

2.6. Statistical analysis

The differences in the studied parameters using the Statistical Analysis System (SAS (2012) program. The least significant difference LSD test was used to compare means. A p value < 0.01 indicated statistical significance.

2. RESULTS AND DISCUSSION

3.1. In vitro cytotoxicity of *Adiantum capillus-veneris* L.

Although advances in the treatment of drug-resistant cancer are the most problematic, it is associated with a worse prognosis for breast cancer. Several research studies examined the effect of medicinal plants against breast cancer because of their good safety and action on target mechanisms [32, 33]. Inactivation of genes encoding the P16 protein has been highlighted in several human cancers [34]. The Michigan Cancer Foundation-7 (MCF-7) is a breast cancer cell line isolated from pleural effusion of an old female; she has primary invasive breast ductal carcinoma positive for estrogen and progesterone receptors [35]. An alcoholic extract of ACV was tested against the breast cell line MCF-7 using the MTT assay. Figure 1 shows the survival of MCF-7 breast cancer cells, untreated and treated with ACV ethanol extract at doses (1000, 500, 250, 125, 62.5) µg/mL for 24 hrs. The highest effect of ACF cytotoxicity was observed at 1000 µg/mL; the data indicated an increase in cytotoxicity of 33.64% compared to 1% in the control group. The IC₅₀ values for the alcoholic extract of ACV were calculated to be 1746 µg/ml (Figure 2); ACV extract has gained considerable attention in cancer treatment. The cytotoxic effect of ACV extract was dependent on the concentration of the extract. The IC₅₀ values for the alcoholic extract of ACV were calculated to be 1746 µg/ml. ACV extracts have gained considerable attention in cancer treatment. There are many secondary metabolites in *Adiantum capillus-veneris*, including flavonoids, triterpenes, phenylpropanoids, carotenoids, zeaxanthin, quercetin, rutin, shikimic acid, and violaxanthin [36].

A study by Ayoub et al aimed to explore the cytotoxic activity of ethanolic extract fractions from the aerial part of ACV against four human cancer cell lines, such as colon, lung, breast, and pancreatic cancer. The data

demonstrated that all the fractions of ethanolic extract showed in vitro inhibition of tumor cell proliferation when tested against different human cancer cell lines. Other reports by Rautray et al demonstrate that ACV extract activity in breast cancer cells is due to its role in modulating proteins involved in the cell cycle and programmed cell death. In addition, ACV effectively modulates the oncogenic molecules and may act as a hopeful agent for cancer therapy. Other studies suggest that *Adiantum pedatum* (AP) extract as a candidate drug may exert its antitumor activity through antioxidant and apoptotic activities [37, 38].

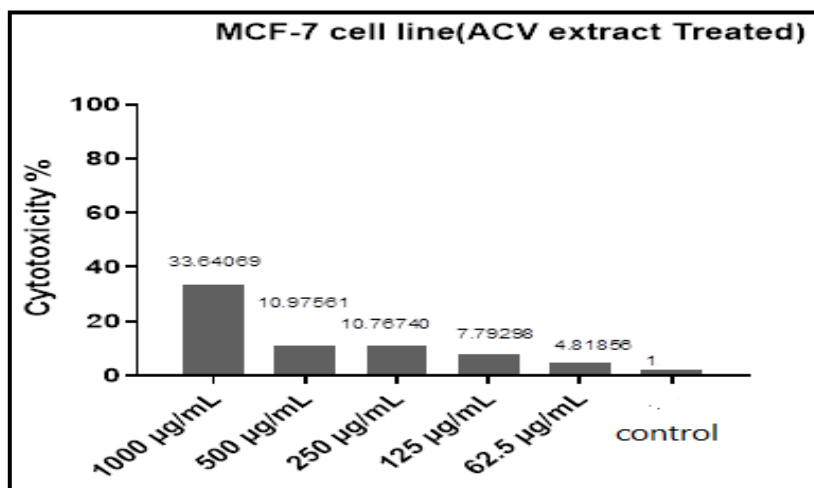


Figure 1. Inhibitory curve showing the effect of ACF on cell viability. The x-axis represents the concentration of the ACV alcoholic extract (in µg), and the y-axis represents the percentage of cell death (Cytotoxicity).

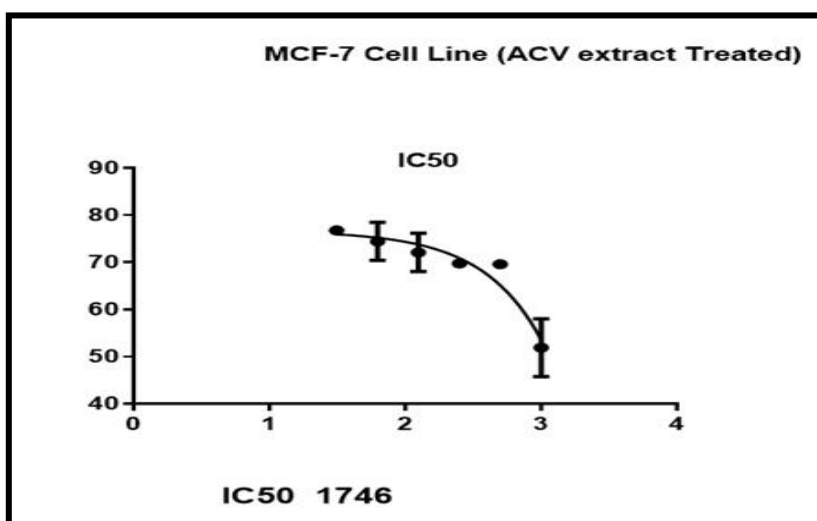


Figure 2. A dose-response curve used to determine the IC₅₀ value of [alcoholic extract of ACV] in MCF-7 cells. The x-axis represents the concentration of the test ACV (in µg), and the y-axis represents the percentage of cell inhibition. The IC₅₀ value is represented by the log of the ACF concentration that inhibits 50% of cell viability.

3.2. The *Adiantum capillus-veneris* L extract effects in *P16* and *Bax* gene expression of MCF -7 breast cancer cell line

Figure 3 shows an example of the qRT-PCR replication of these gene primers, indicating pure and homogeneous melting curves of the studied gene.

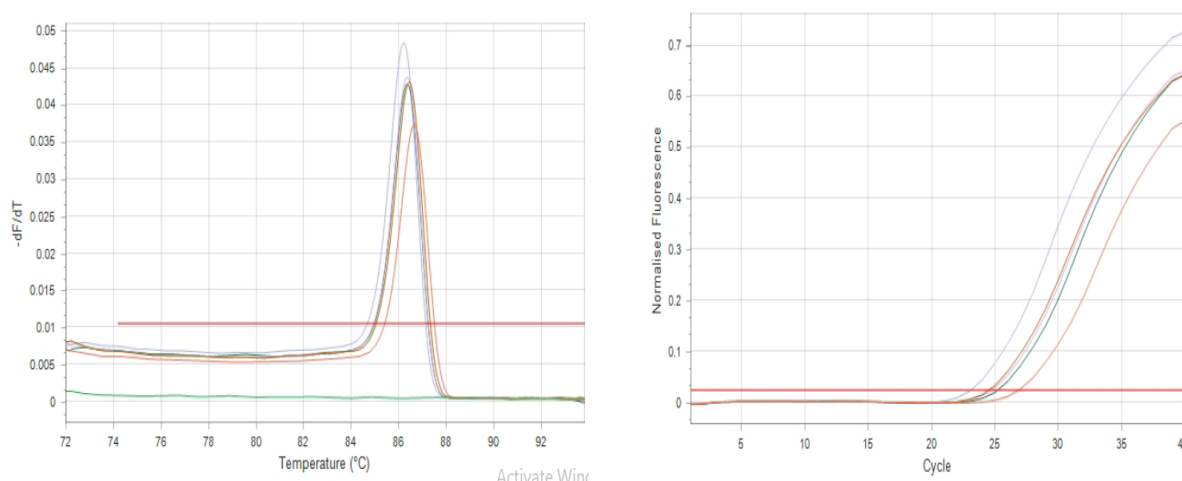


Figure 3. A: Melting curves, B: Cycling curves of gene

3.2.1. The *Adiantum capillus-veneris* L alcoholic extract effects in *P16* gene expression

MCF-7 cells were exposed to ACV extract at the concentrations of the IC₅₀ concentration (1746 $\mu\text{g/mL}$) for 24 hrs. The results show high *P16* gene expression in MCF-7 cells treated with ACV extract compared to the control group (untreated cells). The relative *P16* gene expression increased to 4.087 ± 0.007 in cells treated with the extract compared to (1 ± 0) in control group expression with high significant association (p value < 0.001) (Figure 4). The results refer to high expression of *P16* and *Bax* genes in MCF-7, with a significant increase in relative gene expression to 4.087 for *P16* ($P < 0.001$) and to 1.65 for the *Bax* gene ($p < 0.01$) compared to 1 in the control group. The role of p16 protein is controversial in cell cycle control and carcinogenesis. More than one mechanism has been proposed, with mutations causing upregulation of its gene expression, such as changes in methylation patterns and mutations and deletions [39]. Li et al reported p16 activating proliferation in cervical carcinoma cells through the CDK6-HuR-IL1A axis. Based on the normal functions of the p16 protein, it is expected that p16 protein overexpression reduces the viability of cancer cells. However, when a *p16* gene mutation is linked with the abrogation of the p16 signaling pathway, the Rb signaling will cause overexpression of mutant *p16* in cells, which continue to divide and bypass apoptosis [40, 41]. The pattern of *p16* gene expression varies in breast tumors.

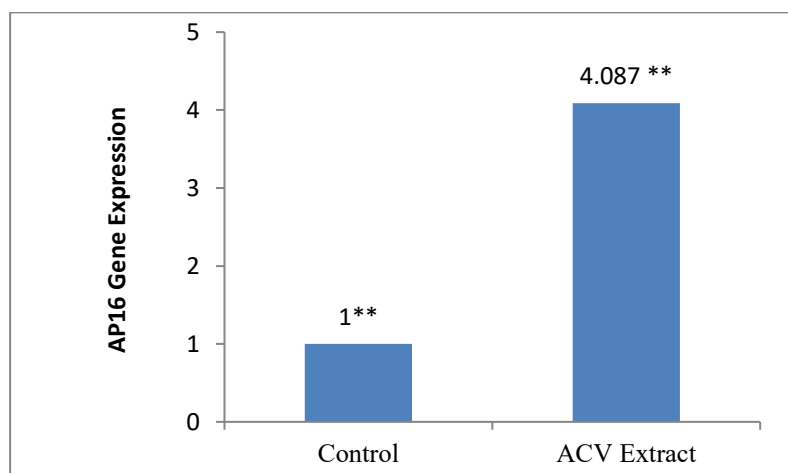


Figure 4. The effect of ACV on the *P16* gene in the MCF7 breast cancer cell line. A high level of *P16* gene expression was induced by ACV extract compared to the control group.

3.3.2. The effects of *Adiantum capillus-veneris* L Extract in *Bax* gene expression

MCF-7 cells were exposed to ACV extract at the concentrations of the IC₅₀ value 1746 $\mu\text{g/mL}$ for 24 hrs. Treated cells showed an increase in *Bax* relative gene expression with a highly significant association at a p value of 0.01. The results refer to a high level of *Bax* gene expression in MCF-7 cells treated with ACV extract (1.650 ± 0.25) in the MCF-7 cell line compared to the control group (non-treated MCF-7 cells) (Figure 5).

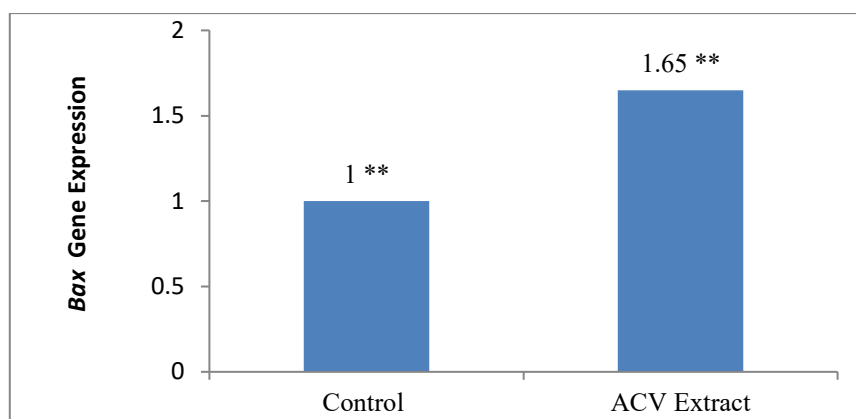


Figure 5. The effect of ACV extract in the *Bax* gene expression in MCF-7 breast cancer cell line, high level of *Bax* gene expression.

Figure 6 showed a comparison of relative gene expression for *P16* and *Bax* genes in MCF-7 cells treated with ACV extract; the results showed a high fold of *P16* gene expression 4.087 ± 0.007 compared to *Bax* gene expression 1.650 ± 0.25 with high significant association ($p < 0.001$). The key gene for programmed cell death (apoptosis) is the *Bax* gene; it leads to the activation of cytochrome C release from mitochondria and activates the apoptotic cascade, and it is the reason for cell death [42]. An increase in the ratio of *Bax* to *Bcl-2* gene expression leads to promoting cell death [43, 44].

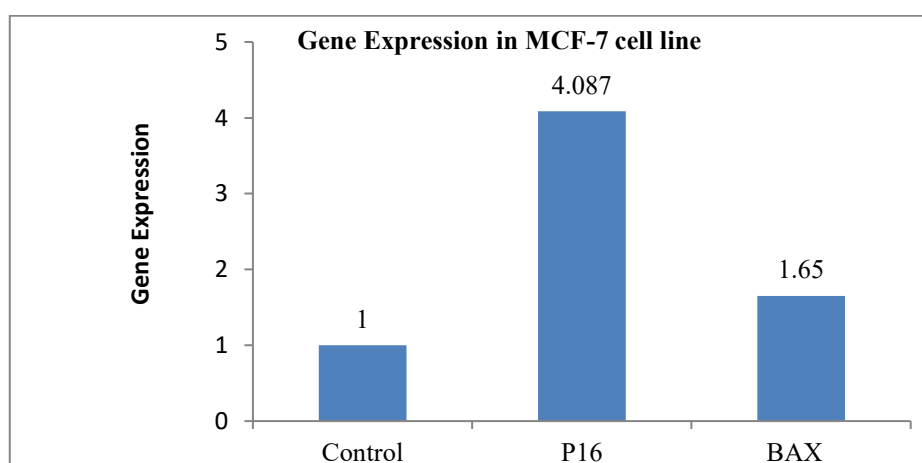


Figure 6. The effect of ACV extract in the *P16* and *Bax* genes expression in MCF-7 breast cancer cell line, low level of *Bax* compared to *P16* fold gene expression.

Other herbal drugs can change the *Bax/Bcl-2* ratio and induce apoptosis in cancer cell lines. Kazemi and Shahrestani found an increase in the *Bax* gene expression rate in AGS (lung cancer cells) when treated with saffron extract, leading to apoptosis. Bagherian and his colleagues reported that Aloe Vera extract inhibited the metabolic process, increased *Bax* expression, decreased *bcl-2* expression, decreased the activity of the MCF-7 cell line, and activated apoptosis in MCF-7 cells. Results indicated that treatment of MCF7 cells with the hydroalcoholic extract of Aloe Vera and Tamoxifen induced apoptosis in cells, so the herbal extract may be promising in cancer treatment [45].

3. CONCLUSION

Adiantum capillus veneris L. Extract could be a promising anticancer agent by modulating the P16 protein in the cell cycle and the pro-apoptotic *Bax* gene and anti-apoptotic protein expression in the MCF-7 breast cancer cell line. Other studies analyze the biochemical composition of ACV and investigate its role in the cell cycle and apoptosis.

ACKNOWLEDGEMENTS

The authors thank the University of Baghdad for its support during this work.

REFERENCES

- [1] World Health Organization, WHO Bacterial Priority Pathogens List, 2024: Bacterial Pathogens of Public Health Importance, to Guide Research, Development, and Strategies to Prevent and Control Antimicrobial Resistance, Geneva, Switzerland: WHO, 2024.
- [2] J. Loscalzo, "Harrison's Principles of Internal Medicine", 2022nd ed. New York, NY, USA: McGraw Hill, (2022).
- [3] F. Mohammadzadeh, and F. Nasri, "P16 expression in human breast carcinoma and its relationship to clinicopathological parameters", *Advanced Biomedical Research*, vol. 12, no. 1, p. 154(2023), DOI: [10.4103/abr.abr.180.22](https://doi.org/10.4103/abr.abr.180.22).
- [4] X. Sheng, Y. Guo, and Y. Lu, "Prognostic role of methylated GSTP1, p16, ESR1 and PITX2 in patients with breast cancer: A systematic meta-analysis under the guideline of PRISMA", *Medicine*, vol. 96, no. 28, p. e7476(2017), DOI: <https://doi.org/10.1097/MD.00000000000007476>.
- [5] J. Ni, "p16INK4A-deficiency predicts response to combined HER2 and CDK4/6 inhibition in HER2+ breast cancer brain metastases", *Nature Communications*, vol. 13, no. 1, p. 1473(2022), DOI: <https://doi.org/10.1038/s41467-022-29081-2>.
- [6] E. Peurala, P. Koivunen, K. M. Haapasaari, R. Bloigu, and A. Jukkola-Vuorinen, "The prognostic significance and value of cyclin D1, CDK4 and p16 in human breast cancer", *Breast Cancer Research*, vol. 15, pp. 1–10(2013), DOI: [10.1186/bcr3376](https://doi.org/10.1186/bcr3376).
- [7] J. Krishnamurthy et al., "p16INK4a induces an age-dependent decline in islet regenerative potential," *Nature*, vol. 443, no. 7110, pp. 453–457(2006), DOI: <https://doi.org/10.1038/nature05092>.
- [8] D. N. Danforth Jr., "Genomic changes in normal breast tissue in women at normal risk or at high risk for breast cancer", *Breast Cancer: Basic and Clinical Research*, vol. 10, pp. BCBRC-S39384, (2016), DOI: <https://doi.org/10.4137/BCBRC.S39384>.
- [9] M. Hassan, O. Feyen, and E. Grinstein, "Fas-induced apoptosis of renal cell carcinoma is mediated by apoptosis signal-regulating kinase 1 via mitochondrial damage-dependent caspase-8 activation", *Analytical Cellular Pathology*, vol. 31, no. 6, pp. 437–456(2009), DOI: <https://doi.org/10.3233/CLO-2009-0488>.
- [10] J. M. Adams, and S. Cory, "The Bcl-2 apoptotic switch in cancer development and therapy", *Oncogene*, vol. 26, no. 9, pp. 1324–1337(2007), DOI: <https://doi.org/10.1038/sj.onc.1210220>.
- [11] T. Khamis, "The antiproliferative activity of Adiantum pedatum extract and/or piceatannol in phenylhydrazine-induced colon cancer in male albino rats: the miR-145 expression of the PI-3K/Akt/p53 and Oct4/Sox2/Nanog pathways", *Molecules*, vol. 28, no. 14, p. 5543(2023), DOI: <https://doi.org/10.3390/molecules28145543>.
- [12] S. K. Ibrahim, "Study the effect of Linum usitatissimum (flax seeds) and Eucalyptus rostrata (Eucalyptus) leaves extracts on Pseudomonas aeruginosa growth in vivo and in vitro", *Biochemical & Cellular Archives*, vol. 20, no. 2, (2020).
- [13] H. Q. Hameed, A. A. Hasan, and R. M. Abdullah, "Effect of Olea europaea L. extraction and TiO₂ nanoparticles against Pseudomonas aeruginosa", *Indian Journal of Public Health Research & Development*, vol. 10, no. 6, (2019).
- [14] A. M. A. Al-Shuwaikh, R. M. A. Al-Shuwaikh, and J. S. Hassan, "Effect of Trigonella foenum extract and ZnO₂ nanoparticles on Some Pathogenic Fungi and Bacteria", *La Prensa Medica*, 105(4), (2019).
- [15] T. A. Muhsen, S. N. Hawar, T. S. Mahdi, and R. Khaleel, "Effect of Eucalyptus and Myrtus extracts identification by gas chromatography-mass spectrometry on some species of Candida as a model of medical plants", *Annals of Tropical Medicine and Public Health*, vol. 23, no. S10, pp. 1–11(2020).
- [16] O. Z. Al-Hayali, M. F. A. Marjani, and A. Maleki, "Evaluation of Rosmarinus officinalis leaves essential oils activity against vancomycin intermediate Staphylococcus aureus (VISA) isolated from Baghdad hospital patients", *Iraqi Journal of Science*, pp. 2153–2167(2023), DOI: <https://doi.org/10.24996/ij.s.2023.64.5.5>.
- [17] K. K. Ghudhaib, and F. M. Khaleel, "Evaluation of antioxidants, antibacterial and antidiabetic activities of aqua-alcoholic marjoram extract", *Baghdad Science Journal*, (2024), DOI: <https://doi.org/10.21123/bsj.2024.9328>.
- [18] H. S. Al-Shmgani, Z. T. Alasady, E. M. Ali, and M. J. Al-Jassani, "Antioxidant, cytotoxicity and phytochemical analysis of Larinus maculatus F. cocoon aqueous extract against lung cancer", in *Journal of Physics: Conference Series*, vol. 1879, no. 2, p. 022025(2021), DOI: [10.1088/1755-1315/421/2/022025](https://doi.org/10.1088/1755-1315/421/2/022025).
- [19] S. M. Fathi, and I. A. Ali, "Cytotoxic effect of the alcoholic extract of Conocarpus erectus leaves on MDA-MB 231 and MCF7 breast cancer cell lines", *Iraqi Journal of Science*, pp. 84–90(2023), DOI: <https://doi.org/10.24996/ij.s.2023.64.1.9>.
- [20] Z. O. Salman, B. M. Alwash, and E. J. Kadhim, "Isolation and identification of flavonoid compounds from Euphorbia milii plant cultivated in Iraq and evaluation of its genetic effects on two types of cancer cell line", *Baghdad Science Journal*, (2024), DOI: <https://doi.org/10.21123/bsj.2024.9276>.
- [21] A. N. Khalaf, and I. J. Abed, "Evaluating the in vitro cytotoxicity of Thymus vulgaris essential oil on MCF-7 and HeLa cancer cell lines", *Iraqi Journal of Science*, pp. 2862–2871(2021), DOI: <https://doi.org/10.24996/ij.s.2021.62.9.3>.
- [22] C. Pan, "Phytochemical constituents and pharmacological activities of plants from the genus Adiantum: A review", *Tropical Journal of Pharmaceutical Research*, vol. 10, no. 5, pp. 681–692(2011), DOI: <https://doi.org/10.4314/tjpr.v10i5.18>.
- [23] J. Gruenwald, T. Brendler, and C. Jaenicke, *PDR for Herbal Medicines*. Montvale, NJ, USA: Medical Economics Company, (2008).
- [24] S. Rautray, S. Panikar, T. Amutha, and A. U. Rajananthini, "Anticancer activity of Adiantum capillus veneris and Pteris quadriureta L. in human breast cancer cell lines", *Molecular Biology Reports*, vol. 45, no. 6, pp. 1897–1911(2018), DOI: <https://doi.org/10.1007/s11033-018-4337-y>.
- [25] Y. C. Wong, M. Y. Ahmad-Mudzaqqir, and W. A. Wan-Nurdiyana, "Extraction of essential oil from cinnamon (Cinnamomum zeylanicum)", *Oriental Journal of Chemistry*, vol. 30, no. 1, p. 37(2014), DOI: <http://dx.doi.org/10.13005/ojc/300105>.
- [26] M. I. Salman, M. A. Emran, and A. M. Al-Shammari, "Spheroid-formation 3D engineering model assay for in vitro assessment and expansion of cancer cells", in *AIP Conference Proceedings*, vol. 2372, no. 1, (2021), DOI: <https://doi.org/10.1063/5.0065362>.
- [27] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays", *Journal of Immunological Methods*, vol. 65, no. 1–2, pp. 55–63(1983), DOI: [https://doi.org/10.1016/0022-1759\(83\)90303-4](https://doi.org/10.1016/0022-1759(83)90303-4).

- [28] S. Wu, and D. K. Shah, "Determination of ADC cytotoxicity in immortalized human cell lines", in *Antibody-Drug Conjugates: Methods and Protocols*, pp. 329–340(2020), DOI: https://doi.org/10.1007/978-1-4939-9929-3_23
- [29] K. J. Livak, "User bulletin no. 2: ABI PRISM 7700 sequence detection system", Applied Biosystems, Foster City, CA, USA, (2001), DOI: <https://doi.org/10.1006/meth.2001.1262>.
- [30] A. Allameh, A. Moazeni-Roodi, I. Harirchi, M. Ravanshad, M. Motiee-Langroudi, A. Garajei, A. Hamidavi, and S. A. Mesbah-Namin, "Promoter DNA methylation and mRNA expression level of p16 gene in oral squamous cell carcinoma: Correlation with clinicopathological characteristics", *Pathology & Oncology Research*, vol. 25, no. 4, pp. 1535–1543(2019), DOI: <https://doi.org/10.1007/s12253-018-0542-1>
- [31] N. K. Rana, P. Singh, and B. Koch, "CoCl₂ simulated hypoxia induce cell proliferation and alter the expression pattern of hypoxia associated genes involved in angiogenesis and apoptosis", *Biological Research*, vol. 52, no. 1, p. 12, (2019), DOI: <https://doi.org/10.1186/s40659-019-0221-z>.
- [32] K. M. Yap, M. Sekar, C. R. Wu, S. H. Gan, S. R. Sani, M. K. Sahu, and Y. S. Wu, "Drug delivery of natural products through nanocarriers for effective breast cancer therapy: A comprehensive review of literature", *International Journal of Nanomedicine*, vol. 16, pp. 7891–7941(2021), DOI: <https://doi.org/10.2147/IJN.S328135>.
- [33] A. Wahi, "Recent updates on nano-phyto-formulations based therapeutic intervention for cancer treatment", *Oncology Research*, vol. 32, no. 1, p. 19(2023), DOI: <https://doi.org/10.32604/or.2023.042228>
- [34] M. K. Rana, A. P. S. Rana, and U. Khera, "Expression of p53 and p16 in carcinoma breast tissue: depicts prognostic significance or coincidence", *Cureus*, vol. 13, no. 11, (2021), DOI: <https://doi.org/10.7759/cureus.19395>.
- [35] A. V. Lee, S. Oesterreich, and N. E. Davidson, "MCF-7 cells—changing the course of breast cancer research and care for 45 years", *Journal of the National Cancer Institute*, vol. 107, no. 7, p. djv073(2015), DOI: <https://doi.org/10.1093/jnci/djv073>.
- [36] R. Vadi, V. Manisha, and K. Swati, "Hansraj (*Adiantum capillus veneris* Linn.): A systematic review on its ethnobotany, phytochemical and pharmacological profile", *International Journal of Ayurveda and Pharma Research*, (2017).
- [37] Z. Ayoub, S. Malik, and A. Mehta, "In vitro and in vivo anti-cancer efficacy of *Adiantum capillus-veneris* L. against some selected human cancer cell lines and on Ehrlich ascites carcinoma mouse model", *Int. J. Pharm. Sci. Drug Res.*, vol. 12, pp. 267–274(2020), DOI: <https://doi.org/10.25004/IJPSDR.2020.120309>
- [38] S. Rautray, S. Panikar, T. Amutha, and A. U. Rajananthini, "Anticancer activity of *Adiantum capillus veneris* and *Pteris quadriureta* L. in human breast cancer cell lines", *Molecular Biology Reports*, vol. 45, no. 6, pp. 1897–1911(2018), DOI: <https://doi.org/10.1007/s11033-018-4337-y>
- [39] K. J. Van Zee, J. E. Calvano, and M. Bisogna, "Hypomethylation and increased gene expression of p16INK4a in primary and metastatic breast carcinoma as compared to normal breast tissue", *Oncogene*, vol. 16, no. 21, pp. 2723–2727(1998), DOI: <https://doi.org/10.1038/sj.onc.1201794>
- [40] M. Li, "p16 promotes proliferation in cervical carcinoma cells through CDK6-HuR-IL1A axis", *Journal of Cancer*, vol. 11, no. 6, p. 1457(2020), DOI: <https://doi.org/10.7150/jca.35479>
- [41] M. Feriancova, I. Walter, C. F. Singer, J. Gazdarica, and K. Pohlodek, "Expression of COX-2, p16, and Ki67 in the range from normal breast tissue to breast cancer", *Neoplasma*, vol. 68, no. 2, pp. 342–351(2021) , DOI: https://doi.org/10.4149/neo_2020_200731N798
- [42] Y. Tsujimoto and S. Shimizu, "Bcl-2 family: life-or-death switch", *FEBS Letters*, vol. 466, no. 1, pp. 6–10(2000), DOI: [https://doi.org/10.1016/S0014-5793\(99\)01761-5](https://doi.org/10.1016/S0014-5793(99)01761-5)
- [43] M. Yadegari, S. Riahy, S. Mirdar, G. Hamidian, and P. Mosadegh Zavaragh, "Effect of the *Adiantum capillus veneris* extract on Bax and Bcl2 apoptotic markers of lung modulation in trained rats and exposed to hypoxic stress", (2017).
- [44] N. Kazemi and S. B. Shahrestani, "Effect of saffron extract on expression of Bax and Bcl-2 genes in gastric adenocarcinoma cell line (AGS)", *Gene, Cell and Tissue*, vol. 5, no. 3, (2018), DOI: <https://doi.org/10.5812/gct.63608>
- [45] T. Bagherian, S. H. Tackallou, and A. Mohammadgholi, "Quantitative measurement of Bax and Bcl2 genes and protein expression in MCF7 cell-line when treated by Aloe Vera extract", *Gene Reports*, vol. 23, p. 101123(2021), DOI: <https://doi.org/10.1016/j.genrep.2021.101123>.