Published online 2025 April **Original Article**

Impact of Iranian Viola Odorata Extract on the Malignant Traits of Colorectal Cancer Cells

Jalil Arab[1], Farzad Seyed Forootan[2,3], Mohammad Armin[4], Saghar Yousefnia[5], Marzieh Nematollahi[2], Iman Niktab[6], Mohammad Hossien Nasr Esfahani[2], Jafar Vatandoost[7], Kamran Ghaedi[5,*]

1. Department of Agricultural Biotechnology, Plant Branch, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran
2. Department of Cellular Biotechnology at Cell Science Research Center, Royan Institute of Biotechnology, ACECR, Isfahan, Iran
3. Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran
4. Department of Agronomy and Plant Breeding, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran
5. Department of Cell and Molecular Biology, Semnan University, Semnan, Iran
6. Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
7. Department of Biology, Hakim sabzevari University, Sabzevar, Iran

*** *Corresponding author:*** Kamran Ghaedi, Department of Cell and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Hezar Jerib Ave., Azadi Sq, Isfahan, Iran. Email: kamranghaedi@sci.ui.ac.ir

Received 2024 April 13**; Accepted** 2025 February 04.

Abstract

Background: Cancer, particularly colorectal cancer, is a growing global public health issue with a significant impact on individuals aged fifty and older. Viola odorata, a traditional herbal remedy, has been investigated for its potential anti-cancer effects, particularly in inhibiting the metastasis of certain cancer cell types.

Objectives: This study aimed to evaluate and compare the effects of Viola odorata extract on the malignant characteristics of colorectal cancer cell lines HT-29 and HCT-116.

Methods: Samples of Viola odorata were collected from five different geographical locations in Iran. The chemical composition of the extracts was analyzed using Fourier transform infrared spectroscopy (FTIR), and the concentration of polyphenols and flavonoids was determined by high-performance liquid chromatography (HPLC). The hydro-alcoholic extract was then used to treat HT-29 and HCT-116 colorectal cancer cell lines. Apoptosis, migration, colony formation, and cell viability were assessed and compared.

Results: Treatment with Viola odorata extract induced apoptosis in both HT-29 and HCT-116 cell lines. The extract also significantly reduced cell migration and colony formation. Cell viability was markedly decreased, with HCT-116 cells showing greater sensitivity to the extract than HT-29 cells.

Conclusion: Viola odorata extract demonstrated anti-cancer activity against colorectal cancer cell lines, with a more pronounced effect in HCT-116 cells. These findings suggest that Viola odorata may be a promising therapeutic agent for targeting colorectal cancer cells, especially at higher concentrations, and its efficacy appears to be cell-dependent.

Keywords: Apoptosis; Colorectal cancer; Flavonoids; Viola odorata

1. Background

Colorectal cancer (CRC) occurs primarily in the colon or the rectum. It plays a crucial role in mortality and cancer complications (1). CRC is the second most common cancer in women, the third in men, and the fourth worldwide (2). When there is an increase in abnormal cellular proliferation and survival potential, these

atypical cells develop benign adenomas that may turn into carcinomas over decades, leading to metastasis (3). The CRCs comprise a highly heterogeneous group of diseases driven by a wide range of mutagens and mutations. Since not all types of CRC share the same stimulatory mutation, it is difficult to define a molecular "catch-all" therapy (4). According to the temporal specifications and demographic projections, the global CRC burden is expected to increase by up to 60% by 2030, with more than 2.2 million new cases and 1.1 million cancer deaths. These characteristics may vary depending on the economic developments of different countries (5). Numerous factors affect CRC, including family history (6), old age (7), excessive alcohol consumption (8), lack of physical activity (9), diabetes, red meat consumption, high-fat diets with no fiber (10), and inflammatory bowel diseases (i.e., ulcerative colitis and Crohn's disease) (11). CRC complications could also be affected by lifestyle, obesity, and diet (12).

Currently, various treatments and prognostic procedures are being developed to combat this type of cancer (13). Viola odorata Linn. (Violaceae), called Banafsha or sweet violet, is a medicinal plant native to Asia, Europe, Australia, and North America (14). V. odorata has multiple therapeutic properties, such as anti-inflammatory (15), anti-rheumatic (16), anti-hypertension (17), anti-cancer (18-22), and antioxidant activity (23). Some active ingredients of V. odorata are salicylic acid derivatives, saponin, alkaloids (e.g., anthocyanidins), violins, and cyclotides (e.g., cycloviolacin) (24). Cyclotides can destroy the membrane at high concentrations in cancer cells via intercalating with membrane-bound sphingomyelin and cholesterol-rich membrane lipid rafts (25). A subset of flavonoids, anthocyanin, activates the apoptosis pathway via the apoptosis induction factor, Caspase 3, and endonuclease G (pro-apoptotic factor). In addition, it prevents the progression and growth of cancer cells via cell cycle arrest in G0/G1 and G2/M by inducing p21 wild-type p53-activated fragment and P27 cyclin-dependent kinase inhibitor one expression and reducing cyclin A and B (26). Chemotherapeutic agents, DNA-damaging drugs (e.g., doxorubicin and cisplatin), antimetabolites (e.g., methotrexate), anti-tubulin agents (e.g., taxis), and hormones have various side effects, including bone marrow suppression, hair loss, drug resistance, cardiac toxicity, neurological dysfunction, and gastric ulcer (27, 28). Most anti-cancer herbs have antioxidant activity through their phenolic compounds. Phenolic compounds (including flavonoids and flavonols) exhibit antioxidant properties because of the hydroxyl groups they contain (29-31). Medicinal plants with antioxidant activities play a crucial role in scavenging free radicals. Mechanisms of antioxidants include quenching free radicals, reducing peroxide, transition metal chelating, and simulating the activities of antioxidant enzymes in vivo (30). An in vivo study has shown that V. odorata extract significantly decreases tumor size of MCF7, SKBR3, and their derived mammospheres (3D) in chicken embryos (20).

Herbs and their extracts might offer a treatment option for CRC with fewer side effects compared to standard treatments, which often entail side effects. The present laboratory study aimed to identify the effects of V. odorata alcoholic extract on migration, apoptosis, colony formation, and cell survival of CRC cell lines and to compare its properties with untreated controls.

2. Objectives

This study aimed to evaluate and compare the effects of Viola odorata extract on the malignant characteristics of colorectal cancer cell lines HT-29 and HCT-116.

3. Methods

3.1. Plant material

In March and April of 2021, during the growing season of the plant, whole aerial parts of V. odorata were collected and

identified using Flora Iranica (31) from five geographical locations in Iran based on the vegetation maps and available information (Ghazvin, Gilan, Golestan, Kermanshah, and Mazandaran). The samples were dried at room temperature (25 °C) in the shade and a ventilated place. The chemical composition of the plants was evaluated using Fourier transform infrared (FTIR) spectroscopy and high-performance liquid chromatography (HPLC) techniques. The samples were kept in the herbarium of Gonbad Kavous University of Technology for further investigations (herbarium code: GKU / NO. 803896).

3.2. Cell culture

Two human CRC cell lines, HT-29 (Genetic Storage Center, Tehran, Iran) and HCT-116 (Pasteur Institute, Tehran, Iran), were purchased. Two cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 1% L-glutamine, and 1% non-essential amino acids at 37 °C in a humidified atmosphere with 5% CO2 (all chemical materials were purchased from Sigma, USA).

3.3. Viola odorata hydro-alcoholic extract

The aerial parts of V. odorata were ground and prepared for extraction. Phenolic and flavonoid extraction was performed based on a modified method described by Gharibi et al. (32). Briefly, 10 g of aerial parts powders were extracted with 200 ml aqueous methanol (80 %, v/v). The extraction was performed with an orbital shaker (150 rpm) for 72 h at 25°C. Then, the extract was filtered using a Whatman filter paper No 40 (15 cm). Finally, the filtered extract was evaporated at 40 °C and dried in desiccators under a vacuum to a constant weight.

3.4. FTIR spectroscopy

FTIR is one of the best tools to identify the types of chemical bonds (functional groups) in compounds. This research used FTIR to characterize the functional groups in the aerial parts of V. odorata, especially hydroxyl (-OH) groups with antioxidant functions. A small amount (5 mg) of aerial parts powder was dispersed in dry potassium bromide. The mixture was thoroughly mixed in a mortar and pressed 6 times in 2 min to form a thin K-Br disk. The disc was then placed in a sample cup of diffuse reflection services. FTIR Model 6300 Japan (JASCO, Tokyo, Japan) recorded the FTIR spectrum of plant materials in the wavelength range of 400-4,000 cm-1. The FTIR spectra were analyzed using the Brangule et al. method. (33).

3.5. HPLC

The V. odorata extract was analyzed by an HPLC system (model Agilent 1090). HPLC washout method has previously been used by Gharibi et al. (34). Pure solutions of recognized ingredients were used as external standards for HPLC analysis. All standards (tannic acid, rosmarinic acid, apigenin, quercetin, rutin hydrate, rutin, ferulic acid, gallic acid, caffeic acid, luteolin, p-coumaric acid, and chlorogenic acid) were optimally dissolved in HPLC grade methanol before injecting into the analytical HPLC system. A 0.22 µm nylon acro-disk filter was used, and 20 µl of the samples were injected. The stationary phase had a 250 mm × 4.6 mm (5 µm) symmetry C18 column (Waters Crop., Milford, MA, USA; 10 mm × 4 mm I.D.), and the mobile phase included solvent A and B with a flow rate of 0.8 mL min−1. The detection was accomplished between 200 and 400 nm through a UV detector. In the mobile phase, 0.1% of water-formic acid was used as solvent A, and 0.1% of formic acid in acetonitrile as solvent B. The gradient conditions were also utilized: a linear step from 10% to 26% solvent B (v/v) for 40 min, 65% solvent B for 70 min, and 100% solvent B for 75 min. The components were identified by comparing the peak area and retention time

by pure standards. In the long run, the results were calculated as mg/100 g of the sample dry weight.

3.6.MTS assay

The MTS assay was used to assess the cell viability of HT-29 and HCT-116 in control (untreated) and treatment conditions. Control cells received only a culture medium. Briefly, the cells were digested in a single-cell suspension and cultured in 96-well polystyrene plates with 5 × 103 cells/100 µL per well. Cells were cultured in normal conditions before treating with different doses of fresh hydro-alcoholic extraction of V. odorata (50, 100, 200, 400, 600, 800, and 1,000 µg/mL) at 37 °C for 24, 48, and 72 h. Afterward, 20 µl of prepared MTS solution (Sigma, USA) was added to each well and incubated at 37 °C for three h. Eventually, the ELISA Plate Reader assessed cell viability under 492 nm wavelength and evaluated survival rates (35). All experimental samples were accomplished in triplicate and compared to the controls.

3.7. Migration assay (scratch assay)

A scratch assay was performed to test the migration capacity of CRC cell lines under control and treatment conditions. Cell lines were cultured in 6-well dishes at a seeding concentration of 2 × 105 cells/well. The treatment group was treated with optimized doses of V. odorata extract. To arrest proliferation, after 48 h, 20 µg/mL mitomycin (Sigma, USA) was added to the dishes with 80-90% confluency for two h. A thin scratch/wound was made with a 100 µL pipette tip. Images were taken by Olympus camera (Japan) attached to an inverted microscope at 40 × magnifications afterward. The distance between the two lines of the wound was measured at 0, 24, and 48 h after scratching to calculate the migration rate. Wound closure (%) was calculated based on the following ratio:

Wound closure percent = AΔh/A0h × 100%.

where A is the gap size (µm) when the scratch was carried out (0 h), while AΔh is the difference in the gap size at the second measurement compared to the initial size of the gap.

3.8. Soft agar assay

A soft agar assay was performed to calculate the colony formation or anchorage-independent proliferation ability of HT-29 and HCT-116 in control and treatment conditions. For this purpose, 4 g of low melting temperature agarose gel (LMTAG) was dissolved in 100 ml of water to prepare the desired gel. After autoclaving, it was placed in the refrigerator to be used in the soft agar assay. The assay was performed in six-well plates that were pre-coated with 2 mL of 1.2% (w/v) LMTAG (Sigma, USA) in a 2x culture medium (high-glucose DMEM supplemented with 20% FBS, 1% penicillin/streptomycin, 1% L-glutamine, and 1% non-essential amino acids) that was solidified at room temperature. Cell lines were then harvested and adjusted to medium to 104 (10,000) cells/mL, which were then incorporated with 1 mL of pre-warm 0.6% (w/v) LMTAG and were located on top of the present basement gel layer. The complex was solidified before incubation at 37 °C in a humidified incubator with 5% CO2 for 3-4 weeks. During this time, the cells were fed with 500 µl culture medium in a well once a week. At the end of the assay, the colonies were stained by adding 500 µl of 30% MTS in the incubator for three hours. Colonies larger than 50 µm were counted (36).

3.9. Apoptosis assay

The apoptotic assay was based on the Annexin V-FITC/PI kit protocol (Sigma, USA). After 48 h treatment with the optimized concentration of V. odorata extract, the cells were detached enzymatically via 0.05% trypsin/EDTA and washed with calcium

buffer. Subsequently, 10 µL Annexin V FITC and 10 µL propidium iodide were added to 100 µL cell suspension in calcium buffer and incubated for 20 min at 4 °C in the dark. Flow cytometry analysis was accomplished by the FACSCalibur flow cytometer (Becton Dickinson, USA) (37).

3.10. Statistical analysis

ImageJ performed statistical analysis for Windows (64-bit Java 1.8.0_172) and GraphPad Prism 8. The results were presented as mean ± SEM for at least three independent experiments for each analysis. Student's t-test (unpaired, two-tailed) with p-values of < 0.05 was used to indicate statistically significant differences.

4. Results

4.1. FTIR analysis

FTIR was used to discover the functional groups in the powder obtained from the aerial parts of V. odorata, which were collected from five locations in Iran (Golestan, Gilan, Ghazvin, Kermanshah, and Mazandaran). A high level of antioxidant compounds (in the form of hydroxyl groups (-OH) in the range of 3,200-3,500 wavenumber cm-1) was observed in the samples. The FTIR spectra of V. odorata are presented in Figures 1A, 1B, and B2. The interpretations of V. odorata compounds are presented in Table 1, Figure 1, and Figure 2.

Table 1. FTIR interpretation of *V. odorata* compounds

Location	Wavenumber cm^{-1} (Test sample)	Wavenumber cm^{-1} (Reference article)	Functional group assignment	Phyto compounds identified
Golestan	3397.96 cm^{-1}	3200-3500	O-H stretch, Hydroxyl group, H-bonded	Poly Hydroxyl compound
	1735.62 cm^{-1}	1705-1765	C=O stretch, Carbonyl	Carbonyl compound
	1647.88 cm^{-1}	1600-1650	C=O stretching vibration, Ketone group	Ketone compounds
	1415.49 cm^{-1}	1400-1500	C=C-C, Aromatic ring stretch	Aromatic compound
	1321.96 cm^{-1}	1310-1410	O-H bend, Alcoholic group	Phenol or tertiary alcohol
	1053.91 cm^{-1}	1070-1150	C-O stretch, Ether group	Cyclic ethers
Gilan	3386.39 cm^{-1}	3200-3500	O-H stretch, Hydroxyl group, H-bonded	Poly Hydroxyl compound
	1735.62 cm^{-1}	1705-1765	C=O stretch, Carbonyl	Carbonyl compound
	1651.73 cm^{-1}	1600-1650	C=O stretching vibration, Ketone group	Ketone compounds
	1417.42 cm^{-1}	1400-1500	C=C-C, Aromatic ring stretch	Aromatic compound
	1321 cm^{-1}	1310-1410	O-H bend, Alcoholic Group	Phenol or tertiary alcohol
	1049.09 cm^{-1}	1070-1150	C-O stretch, Ether group	cyclic ethers
Kermanshah	3391.21 cm^{-1}	3200-3500	O-H stretch, Hydroxyl group, H-bonded	Poly Hydroxyl compound
	1735.62 cm^{-1}	1705-1765	C=O stretch, Carbonyl	Carbonyl compound
	1650.77 cm^{-1}	1600-1650	C=O stretching vibration, Ketone group	Ketone compounds
	1419.35 cm^{-1}	1400-1500	C=C-C, Aromatic ring stretch	Aromatic compound
	1321.96 cm^{-1}	1310-1410	O-H bend, Alcoholic group	Phenol or tertiary alcohol
	1055.84 cm^{-1}	1070-1150	C-O stretch, Ether group	Cyclic ethers
Mazandaran	3380.6 cm^{-1}	3200-3500	O-H stretch, Hydroxyl group, H-bonded	Poly Hydroxyl compound
	1735.62 cm^{-1}	1705-1765	C=O stretch, Carbonyl	Carbonyl compound
	1649.8 cm^{-1}	1600-1650	C=O stretching vibration, Ketone group	Ketone compounds
	1415.49 cm^{-1}	1400-1500	C=C-C, Aromatic ring stretch	Aromatic compound
	1321.96 cm^{-1}	1310-1410	O-H bend, Alcoholic Group	Phenol or tertiary alcohol
	1052.94 cm^{-1}	1070-1150	C-O stretch, Ether group	Cyclic ethers
Ghazvin	3389.28 cm^{-1}	3200-3500	O-H stretch, Hydroxyl group, H-bonded	Poly Hydroxyl compound
	1734.66 cm^{-1}	1705-1765	C=O stretch, Carbonyl	Carbonyl compound
	1650.77 cm^{-1}	1600-1650	C=O stretching vibration, Ketone group	Ketone compounds
	1416.46 cm^{-1}	1400-1500	C=C-C, Aromatic ring stretch	Aromatic compound
	1321.96 cm^{-1}	1310-1410	O-H bend, Alcoholic Group	Phenol or tertiary alcohol
	1053.91 cm^{-1}	1070-1150	C-O stretch, Ether group	Cyclic ethers

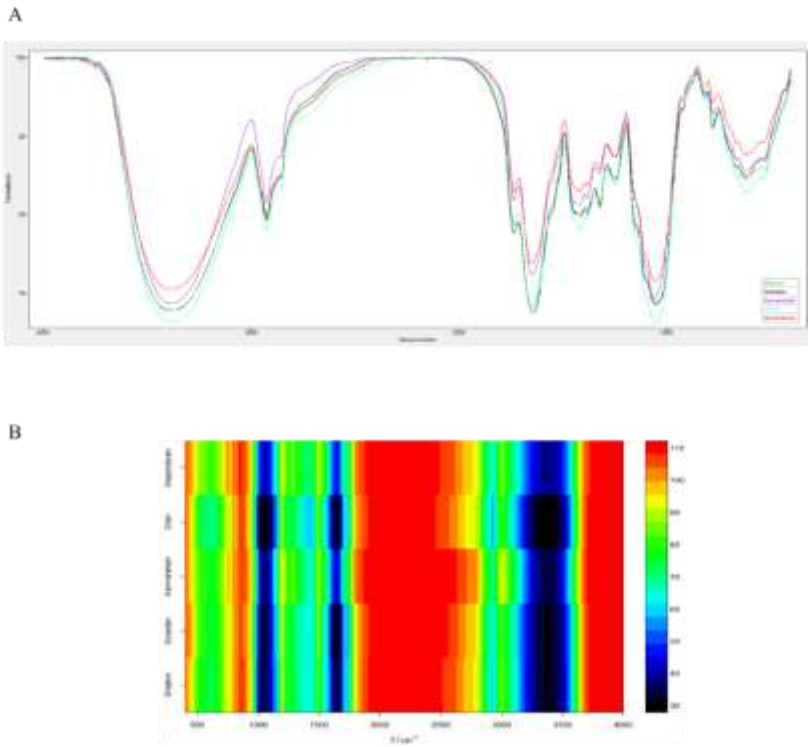

Figure 1: FTIR spectrum (A) and FTIR heatmap (B) of *V. odorata* from five locations (Golestan, Gilan, Ghazvin, Kermanshah, and Mazandaran). FTIR was used to discover the functional groups in the powder obtained from the aerial parts of *V. odorata*. A high level of antioxidant compounds (in the form of hydroxyl groups (-OH) in the range of 3,200-3,500 wavenumber cm^{-1}) was observed in the samples.

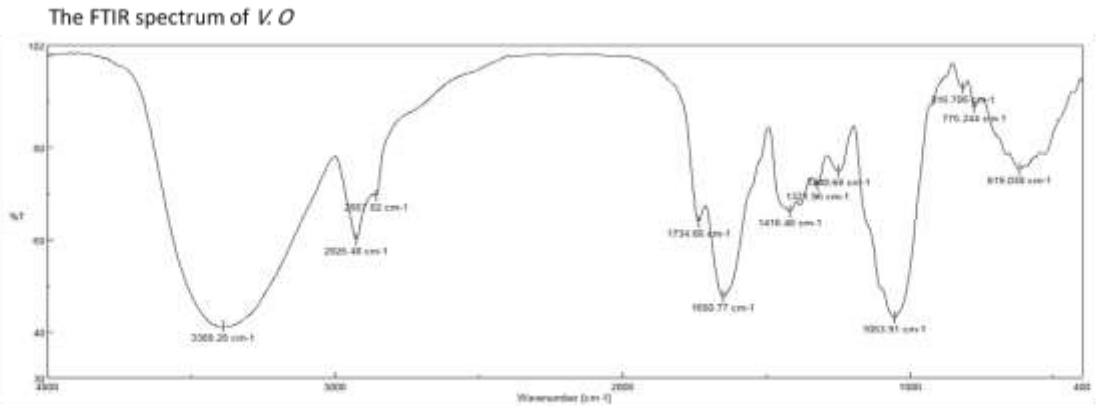

Figure 2: FTIR spectrums of *V. odorata* of Mazandaran location

4.2. HPLC of hydro-alcoholic extraction of Viola odorata

Quantitative characterization of phenolic and flavonoid compounds of V. odorata was carried out using HPLC analysis at 266 and 330 nm. Among five locations, the samples from Mazandaran showed the highest levels of phenolic and flavonoid compounds. The profiles of the HPLC peaks from the Mazandaran sample are shown in Table 2. HPLC results of the other locations were presented in a supplementary figure. The HPLC detected the presence of 12 flavonoid and phenolic compounds. All these compounds are displayed as mg/100 g dry weight of the sample. The most polyphenolic components of V. odorata were gallic acid, chlorogenic acid, caffeic acid, rosmarinic acid, tannic acid, rutin hydrate, and rutin (Figure 3).

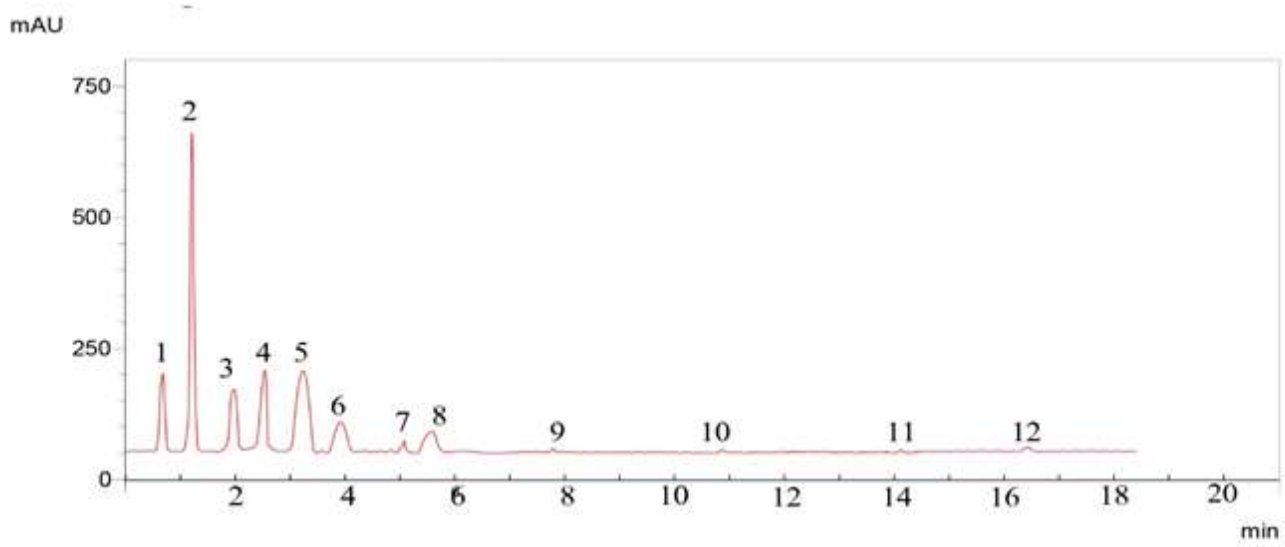

Figure 3: HPLC chromatogram of *V. odorata* of Mazandaran location. The polyphenolic compounds were characterized in the footnote.

1: tannic acid, 2: gallic acid, 3: chlorogenic acid, 4: caffeic acid, 5: rutin hydrate, 6:rutin, 7: p-coumaric acid, 8: ferulic acid, 9: rosmarinic acid, 10: luteolin, 11: quercetin, 12: apigenin

Among phenolic acids, gallic acid contained the highest content (14 mg/100 g), while rutin had the highest values among flavonoids (3.93 mg/100g).

Table 2. Based on HPLC analysis, the major phenolic and flavonoid compounds of V. odorata are from the Mazandaran location. The values are expressed in mg/100 g of sample dry weight. [a] The data were sorted based on components' retention time (RT).

Standards	RT (min)	mg/100g sample
Tannic acid	0.67	6.9
Gallic acid	1.19	14
Chlorogenic acid	1.97	18.1
Caffeic acid	2.42	0.02
Rutin hydrate	3.14	0.27
Rutin	3.96	3.93
p-Coumaric acid	5.04	.82
Ferulic acid	5.64	0.20
Rosmarinic acid	7.87	1.4
Luteolin	10.92	0.01
Quercetin	14.12	0.005
Apigenin	16.48	0.03

4.3.Viola odorata extract induced cell death in HT-29 and HCT-116

MTS assay was performed to recognize the cytotoxic potential (50% inhibitory concentration (IC50)) of V. odorata extract on HT-29 and HCT-116. Cells were treated with an increasing concentration of plant extract (0-1,000 µg / ml) for 24, 48, and 72 h. Although after 24 h, cell death was only significant at the dose of 1,000 µg/mL, increasing doses of V. odorata extract demonstrated that after 48 and 72 h, cell viability decreased in a dose- and time-dependent manner. The value of IC50 was 800 µg/mL for HT-29 and HCT-116 ($P<0.05$). HT-29 and HCT-116 exhibited some resistance at 600 µg/mL concentrations. Based on these results, we proposed that V. odorata extract could induce cell death in CRC cell lines in a cell-dependent manner (Figure 4).

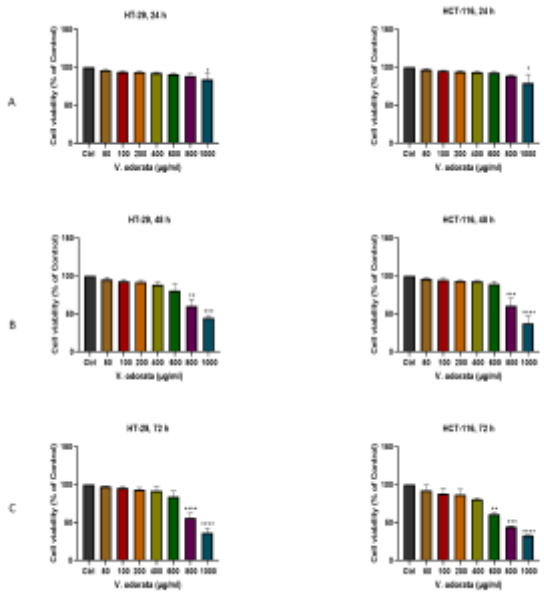

Figure 4: Cytotoxic activity of *V. odorata* extracts on HCT-116 and HT-29. Various concentrations of *V. odorata* extract were used to treat the cell lines in A) 24 h, B) 48 h, and C) 72 h. The effective concentration of *V. odorata* extract was 800 µg/mL for HCT-116 and HT-29. Stars indicate significant differences between samples and control (0 µg/mL) at **p* < 0.05, ***p* < 0.01, ****p* < 0.001

4.4.Viola odorata extract induced apoptosis in HT-29 and HCT-116

Annexin V kit was utilized to detect the apoptosis rate (early and late apoptosis) in HT-29 and HCT-116. The results demonstrated that optimized doses of V. odorata extract (800 µg/ mL for HT-29 and HCT-116) induced 13.15% and 25.13% early apoptosis and 17.44% and 15.42% late apoptosis in HT-29 HCT-116, respectively (P<0.05). Data showed that V. odorata extract induced apoptosis in a cell-dependent manner in CRC cell lines (Figures 5A, B). Some necrosis was also observed in both treated and untreated groups in all cell lines (Figure 5A).

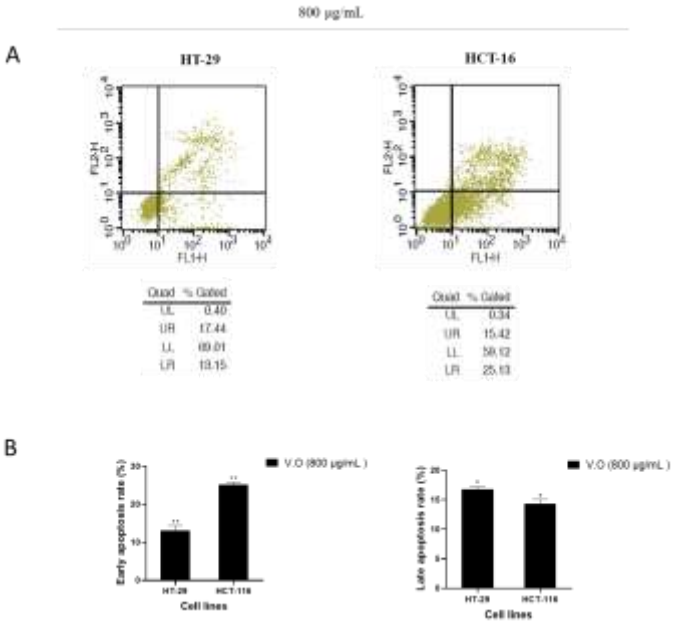

Figure 5:. Detection of apoptosis activity of *V. odorata* extracts on HT-29 and HCT-116. A) Annexin V cytometry analysis for the detection of apoptosis rate in HT-29 and HCT-116, B) Data showed that *V. odorata* extract induced early and late apoptosis in HT-29 and HCT-116 (P<0.01). **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

4.5. *Viola odorata extract inhibited the migration of HT-29 and HCT-116.*

A wound-healing assay at 0, 24, 48, and 72 h after scratching was performed and reported as migration rates (Figures 6A, B). The migration rates of HT-29 and HCT-116 treated with V. odorata extracts were 21% and 35%, respectively, 24 hours after scratching. This rate was approximately 9% and 11% lower than the migration rates of the control groups without treatment (P<0.05). Gradually, 48 h after scratching, the migration rates of HT-29 and HCT-116 treated with plant extracts were 18% and 10%, respectively, which showed a decrease of 19% and 9%, respectively, compared to the related untreated samples (P<0.05) (Figures 6C, D). The migration rates of treated HT-29 and HCT-116 were around 8% and 9%, respectively, 72 h after scratching, demonstrating a 21% and 17% reduction in comparison to their untreated control groups (P<0.01) (Figures 6C, D). Overall, the data showed that V. odorata extract significantly inhibited the migration of HCT-116 and HT-29. In addition, this inhibition was higher in the migration rate of HCT-116 in comparison to HT-29 (P<0.05).

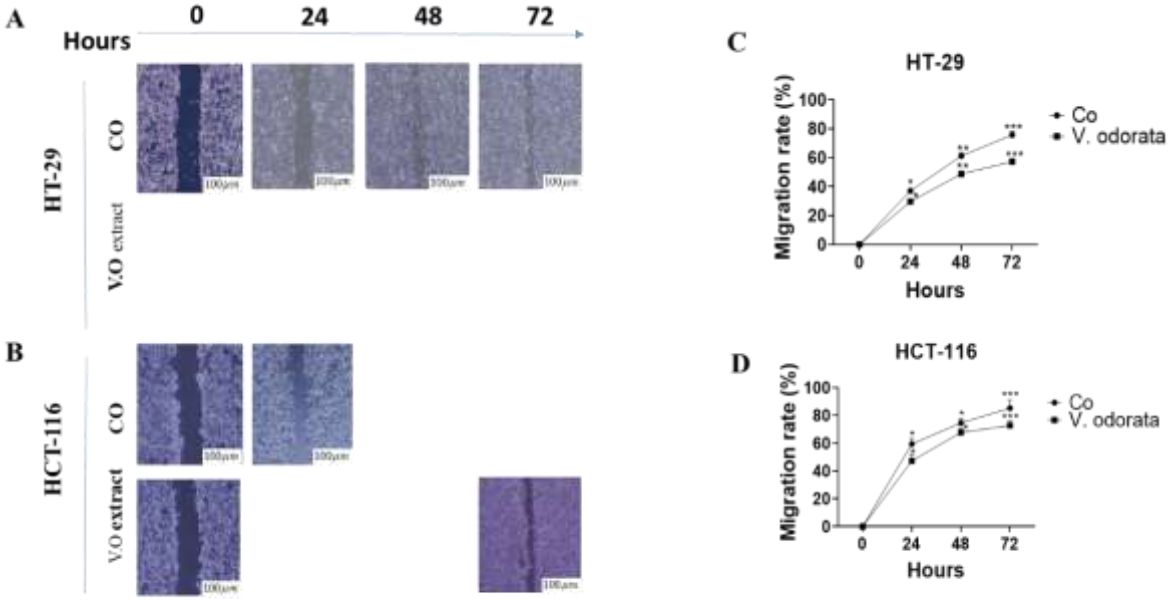

Figure 6: Detection of migration ability of HT-29 and HCT-116 treated by *V. odorata* extract. A series of images of scratch assay from HT-29 and HCT-116 were taken at 0, 24, 48, and 72 h post scratch in both control and treated samples. The groups were A) HT-29 and B) HCT-116. The quantification of the migration rate of monolayers in both control and treated samples was calculated. The groups were C) HT-29 and D) HCT-116. As shown, the migration rate of treated groups in all types of cells was significantly lower than their control counterparts at 24, 48, and 72 h (P<0.05). Results showed that *V. odorata* extract significantly inhibited the migration of HT-29 and HCT-116. Represented value bars are the mean of triplicate independent experiments ± SEM. The bar is 100 µm. Star indicates a significant difference between samples and controls (*V. odorata* untreated) at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

4.6.Viola odorata extract inhibited colony formation of HT-29 and HCT-116

Soft agar assay assessed the colony formation ability of HT-29 and HCT-116 in treated and control groups (Figures 7A, B). Compared to the control groups without treatment, the HT-29 and HCT-116 groups treated with *V. odorata* extract had 2.3% and 2.7% fewer colonies, respectively. This finding showed that V. *odorata* extract significantly reduced the ability of HT-29 and HCT-116 to form colonies. Notably, the reduction was significantly higher in HCT-116 compared to HT-29 cell lines (P<0.05) (Figure 7B).

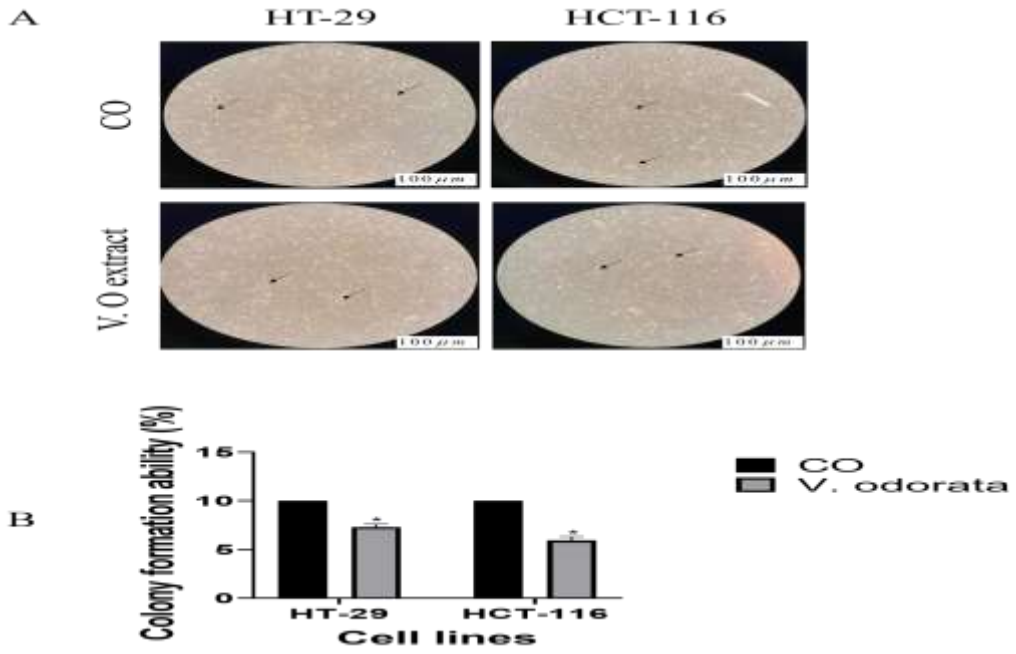

Figure 7: Detection of colony formation ability of HT-29 and HCT-116 treated by *V. odorata* extract. A) Colonies of HT-29 and HCT-116 in control and treatment conditions in soft agar. B) Colony formation ability of HT-29 and HCT-116 in control and treatment conditions in soft agar. *V. odorata* extract decreased the colony formation ability of HT-29 and HCT-116. The bar is 100 µm. Star indicates a significant difference between samples and related controls (*V. odorata* untreated) at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

5. Discussion

As the prevalence of CRC is increasing throughout the world, it is crucial to find effective drugs and treatments. Although the rise in early-onset CRC is unknown, it may be due to dietary changes or other lifestyle factors. These are usually first reflected in the cancer incidence in the younger age group (38). The average survival time for advanced CRC patients is reported to be 18 months (36). The screening methods usually rely on the diagnosis of adenomatous polyps as the precursor lesions of colon cancer to prevent colorectal cancer (39). Standard cancer treatment is generally based on chemotherapy, cytotoxic drugs, radiotherapy, and surgery (40). Chemotherapy has numerous side effects, such as muscle aches, fatigue, stomach pain, headache, diarrhea and vomiting, blood disorders, sore throat, constipation, memory impairment, nervous system damage, loss of appetite, and hair loss (41). However, it has been reported that herbal extracts can stop cancer cells at different stages, such as phases G2 / M and G1 / S, and prevent their growth and proliferation with fewer side effects (42). Viola odorata is an important herb used in cancer treatment due to its anti-cancer compounds, including phenol, cyclotide, and anthocyanin (17). The present study investigated the anti-cancer properties of V. odorata extract in CRC cell lines. Our results showed that V. odorata extract caused cell death, induced apoptosis, and reduced the rate of migration and colony formation in CRC cell lines.

This research used FTIR to identify functional groups in aerial parts of V. odorata. FTIR spectroscopy revealed the composition of functional components in different parts of plants and even assessed their quality (43). The active anti-cancer compounds of V. odorata include cyclotides, saponin, cycloviolacin, salicylic acid derivatives, glycosides, vioacercitin, alkaloids, violins, flavonoids, and anthocyanidins (20).

The current research detected 12 flavonoid and phenolic compounds using HPLC. The most polyphenolic compounds of V. odorata were gallic acid, chlorogenic acid, caffeic acid, rosmarinic acid, tannic acid, rutin hydrate, and rutin. HPLC results have shown the detection of several flavonoids, such as quercetin,

luteolin, kaempferol, and apigenin, in V. odorata extract (20, 44). Some reports have confirmed the anti-cancer effects of flavonoids (45). Several studies have also confirmed the anti-cancer activity of apigenin and luteolin detected in V. odorata (46). Recent reports have displayed that gallic acid, ferulic acid, p-coumaric acid, and syringic acid strongly correlate with free radical scavenging activity (47). The position of the hydroxyl group in polyphenols is mainly responsible for their antioxidant activity; therefore, phenolics may be involved in inhibiting radicals. Similarly, other reports have illustrated a higher antioxidant activity in caffeic and chlorogenic acids than in other phenolic compounds (48). Flavonoids have three rings, named A, B, and C. Numerous studies have shown that the catechol (B) ring in catechins, flavonols, and flavones is the leading cause of their antioxidant activity (49).

Previous studies have demonstrated that the alcoholic extract of V. odorata induced cell death in both cell lines and mammospheres derived from the cell line without any toxic effects on normal mammary cells. This statement indicates that V. odorata extract may induce cell death in a cell-dependent manner based on the cell membrane composition (20). Some studies have illustrated that the cytotoxic effects of V. odorata are induced by the isolation of cyclopeptides in various cancer cell lines (varv F, varv A, and cycloviolacin O2) (50).

Our data from annexin V analysis demonstrated that optimized doses of V. odorata extract induced cell death through early and late apoptosis in HT-29 and HCT-116. In addition, we observed some necrosis in CRC cell lines in both treated and untreated groups. Previous data from annexin V analysis displayed that V. odorata extract at its optimal concentration may cause cell death via early and late apoptosis in SKBR3, MCF7, and their derived mammospheres. It also induced apoptosis by enhancing the activity of caspase 3/7 and 8. Necrosis was also observed in SKBR3 and MCF7cell lines and their derived mammospheres in both groups (treated and untreated). The necrosis could be due to programmed necrosis in cancer cells caused by limited nutrients and insufficient supplies of cancer cells (20).

The necrosis may have also occurred due to late apoptosis (51). Yi et al. illustrated that kaempferol, a natural flavonoid, can induce apoptosis by reducing the expression of Bcl2 and poly ADP ribose polymerase (PARP) and increasing Bax expression and PARP cleavage in breast cancer cells (52). In addition, quercetin (a flavonoid found in extracting V. odorata can lead to apoptosis in breast cancer cells via Foxo3a signaling modification (20).

Tumor metastasis is the leading cause of cancer mortality worldwide (53). Our data showed that V. odorata extract significantly reduced the rate of migration and the wound-healing ability of HCT-116 and HT-29. Similarly, Zeinoddini et al. demonstrated the anti-metastatic effect of V. odorata since the migration rate and wound healing ability of T47-D cells were significantly reduced at the concentration of 1 mg/ml of V. odorata. This efficacy can be explained by various mechanisms, such as the role of Gβγ in preventing breast cancer cell metastasis (54). In addition, NF-κB interference by p53-ROS may reduce the migration of breast cancer cells and subsequently inhibit MMP-2 and MMP-9 gene expression (55). The role of junctional adhesion molecule-A through RAP1 GTPase and β1-integrin in migration and reducing adhesion of breast cancer cells (56), as well as the role of Notch1 in tumor diffusion, proliferation, and metastasis (57), may also be related to the anti-immigration effect of V. odorata. Additionally, in the soft agar assay, we observed that the inhibitory effects of V. odorata extract on the colony formation rate of CRC cell lines varied depending on the cell type, similar to the observations reported by Tuasha et al. (58).

6. Conclusion

In the present study, we investigated the inhibitory activity of V. odorata extract against human HT-29 and HCT-116 CRC cell lines. This study is the first to investigate the effect of V. odorata extract on malignant characteristics of CRC in these cell lines. The lowest dose of the introduced drug with the highest mortality rate was compared in CRC cell lines (treated) and control (untreated) cells. Finally, we proposed V. odorata extract as an anti-cancer drug because it reduces migration and colony formation and increases the apoptosis rate in CRCs. In addition, we speculated that V. odorata might act in a cell-dependent manner. However, further studies are needed to establish this drug's exact mechanism of action. Our study's results reinforce traditional medicine beliefs that V. odorata may be a relative cancer inhibitor.

Acknowledgments: We thank our great colleague, Mr. Abbas Kiani Esfahani, for his technical assistance with flow cytometry.

Availability of data and materials: Raw data and materials are available upon request.

Conflicts of interests: No conflict of interest.

Consent for publication: Not applicable.

Ethics approval and consent to participate: T The permission to use cancer cell lines based on the standard protocols was obtained from the Ethics Committee of Royan Institute (ethics code: IR.ACECR.ROYAN.REC). The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Financial disclosure: No funding information.

Author contributions: All authors contributed equally to this manuscript's research, writing, and revision**.**

Abbreviations: Bcl-2, B-cell lymphoma 2; CRC, Colorectal cancer; DMEM, Dulbecco's modified Eagle's medium; EDTA, Ethylene diamine tetra acetic acid; FBS, Fetal bovine serum; FTIR, Fourier transform infrared spectroscopy; Gβδ, G beta-gamma complex; JAM-A, Junctional adhesion molecule-A; HPLC, High-performance liquid chromatography; IC50, 50% Inhibitory concentration; LMTAG, Low melting temperature agarose gel; MMP, Matrix metalloproteinases; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; PARP, poly ADP ribose polymerase, p27KIP1, p27 cyclin-dependent kinase inhibitor 1; p21WAF1, p21 wild-type p53-activated fragment**.**

References

1. Network CGA. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487(1):330. https://doi.org/10.1038/nature11252PMid:22810696 PMCid: PMC3401966
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(1):69-90. https://doi.org/10.3322/caac.20107PMid:21296855
3. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. N Engl J Med. 1988;319(1):525-32. https://doi.org/10.1056/NEJM198809013190901PMid:2841597
4. Sideris M, Papagrigoriadis S. Molecular biomarkers and classification models in the evaluation of the prognosis of colorectal cancer. Anti-cancer Res. 2014;34(1):2061-8.
5. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin D, Forman D, Bray F. GLOBOCAN 2012 v1.0: Cancer incidence and mortality worldwide: IARC CancerBase 11. Int J Cancer. 2013.
6. Johns LE, Houlston RS. A systematic review and meta-analysis of familial colorectal cancer risk. Am J Gastroenterol. 2001;96(1):2992-3003. https://doi.org/10.1111/j.1572-0241.2001.04677.xPMid:11693338
7. Schultz DJ, Wickramasinghe NS, Ivanova MM, Isaacs SM, Dougherty SM, Imbert-Fernandez Y, Cunningham AR, Chen C, Klinge CM. Anacardic acid inhibits estrogen receptor α-DNA binding and

reduces target gene transcription and breast cancer cell proliferation. Mol Cancer Ther. 2010;9(1):594-605. https://doi.org/10.1158/1535-7163.MCT-09-0978PMid:20197399 PMCid: PMC2837512
8. Huxley RR, Ansary-Moghaddam A, Clifton P, Czernichow S, Parr CL, Woodward M. The impact of dietary and lifestyle risk factors on risk of colorectal cancer: a quantitative overview of the epidemiological evidence. Int J Cancer. 2009;125(1):171-80. https://doi.org/10.1002/ijc.24343PMid:19350627
9. Watson AJ, Collins PD. Colon cancer: a civilization disorder. Dig Dis. 2011;29(1):222-8. https://doi.org/10.1159/000323926PMid:21734388
10. Meyerhardt JA, Catalano PJ, Haller DG, Mayer RJ, Macdonald JS, Benson AB III, Fuchs CS. Impact of diabetes mellitus on outcomes in patients with colon cancer. J Clin Oncol. 2003;21(1):433-40. https://doi.org/10.1200/JCO.2003.07.125PMid:12560431
11. Terzić J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology. 2010;138(1):2101-14. e5. https://doi.org/10.1053/j.gastro.2010.01.058PMid:20420949
12. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(1):683-91. https://doi.org/10.1136/gutjnl-2015-310912PMid:26818619
13. Qudayr A, Kabel AM, AbdElmaaboud MA, Alghamdi WY, Alghorabi A. Colorectal cancer: new perspectives. J Cancer Res Treat. 2018;6(1):80-3.
14. Erhatić R, Vukobratović M, Peremin Volf T, Židovec V. Morphological and chemical properties of selected sweet violet populations. J Cent Eur Agric. 2010;11(1):55-64. https://doi.org/10.5513/JCEA01/11.1.803
15. Koochek M, Pipelzadeh M, Mardani H. The effectiveness of Viola odorata in the prevention and treatment of formalin-induced lung damage in the rat. J Herbs Spices Med Plants. 2003;10(1):95-103. https://doi.org/10.1300/J044v10n02_11
16. Anca T, Philippe V, Ilioara O, Mircea T. Composition of essential oils of Viola tricolor and V. arvensis from Romania. Chem Nat Compd. 2009;45(1):91-2. https://doi.org/10.1007/s10600-009-9244-y
17. Siddiqi HS, Mehmood MH, Rehman NU, Gilani AH. Studies on the antihypertensive and antidyslipidemic activities of Viola odorata leaves extract. Lipids Health Dis. 2012;11(1):1-12. https://doi.org/10.1186/1476-511X-11-6PMid:22233644 PMCid:PMC3286389
18. Gerlach SL, Rathinakumar R, Chakravarty G, Göransson U, Wimley WC, Darwin SP, Mondal D. Anti-cancer and chemosensitizing abilities of cycloviolacin O2 from Viola odorata and psyle cyclotides from Psychotria leptothyrsa. Pept Sci. 2010;94(1):617-25. https://doi.org/10.1002/bip.21435PMid:20564026
19. Lindholm P, Göransson U, Johansson S, Claeson P, Gullbo J, Larsson R, Bohlin L, Backlund A. Cyclotides: A novel type of cytotoxic agents. Mol Cancer Ther. 2002;1(1):365-9.
20. Yousefnia S, Naseri D, Seyed Forootan F, Tabatabaeian M, Moattar F, Ghafghazi T, Nasr Esfahani M, Ghaedi K. Suppressive role of Viola odorata extract on malignant characters of mammosphere-derived breast cancer stem cells. Clin Transl Oncol. 2020;22(1):1619-34. https://doi.org/10.1007/s12094-020-02307-9PMid:32056127
21. Alipanah H, Bigdeli MR, Esmaeili MA. Inhibitory effect of Viola odorata extract on tumor growth and metastasis in 4T1 breast cancer model. Iran J Pharm Res. 2018; 17:276.
22. Deldadeh N. Evaluation and comparison of antimicrobial and anti-cancer effects of aqueous and ethanolic extracts of violet. Multidiscip Cancer Investig. 2022; 6:0-0.
23. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR. Antioxidant and free radical scavenging activity of H. officinalis L. var. angustifolius, V. odorata, B. hyrcana, and C. speciosum. Pak J Pharm Sci. 2010; 23:29-34.
24. Saether O, Craik DJ, Campbell ID, Sletten K, Juul J, Norman DG. Elucidation of the primary and three-dimensional structure of the uterotonic polypeptide kalata B1. Biochemistry. 1995;34(14):4147-58. https://doi.org/10.1021/bi00013a002PMid:7703226
25. Duke JA. Handbook of medicinal herbs. 2nd ed. CRC Press; 2002. https://doi.org/10.1201/9781420040463
26. Li D, Wang P, Luo Y, Zhao M, Chen F. Health benefits of anthocyanins and molecular mechanisms: update from recent decade. Crit Rev Food Sci Nutr. 2017;57(8):1729-41. https://doi.org/10.1080/10408398.2015.1030064PMid:26192537
27. Svangård E, Burman R, Gunasekera S, Lövborg H, Gullbo J, Göransson U. Mechanism of action of cytotoxic cyclotides: cycloviolacin O2 disrupts lipid membranes. J Nat Prod. 2007;70(4):643-7. https://doi.org/10.1021/np070007vPMid:17378610
28. Asadi-Samani M, Kooti W, Aslani E, Shirzad H. A systematic review of Iran's medicinal plants with anti-cancer effects. J Evid Based Complementary Altern Med. 2016;21(2):143-53. https://doi.org/10.1177/2156587215600873PMid:26297173
29. Nussbaumer S, Bonnabry P, Veuthey JL, Fleury-Souverain S. Analysis of anticancer drugs: a review.

Talanta. 2011;85(5):2265-89. https://doi.org/10.1016/j.talanta.2011.08.034 PMid:21962644
30. Dropcho EJ. The neurologic side effects of chemotherapeutic agents. Continuum (Minneap Minn). 2011;17(1):95-112. https://doi.org/10.1212/01.CON.0000394676.67372.87 PMid:22810790
31. Rechinger KH. Flora Iranica. 1963.
32. Gharibi S, Tabatabaei BES, Saeidi G, Goli SAH, Talebi M. Total phenolic content and antioxidant activity of three Iranian endemic Achillea species. Ind Crops Prod. 2013; 50:154-8. https://doi.org/10.1016/j.indcrop.2013.07.038
33. Brangule A, Šukele R, Bandere D. Herbal medicine characterization perspectives using advanced FTIR sample techniques-diffuse reflectance (DRIFT) and photoacoustic spectroscopy (PAS). Front Plant Sci. 2020; 11:356. https://doi.org/10.3389/fpls.2020.00356 PMid:32362902 PMCid:PMC7182013
34. Gharibi S, Tabatabaei BES, Saeidi G, Talebi M, Matkowski A. The effect of drought stress on polyphenolic compounds and expression of flavonoid biosynthesis related genes in Achillea pachycephala Rech. f. Phytochemistry. 2019; 162:90-8. https://doi.org/10.1016/j.phytochem.2019.03.004 PMid:30875522
35. Zhang LC, Jin X, Huang Z, Yan ZN, Li PB, Duan RF, Feng H, Jiang JH, Peng H, Liu W Protective effects of choline against hypoxia-induced injuries of vessels and endothelial cells. Exp Ther Med. 2017; 13:2316-24. https://doi.org/10.3892/etm.2017.4276 PMid:28565844 PMCid:PMC5443310
36. Horibata S, Vo TV, Subramanian V, Thompson PR, Coonrod SA. Utilization of the soft agar colony formation assay to identify inhibitors of tumorigenicity in breast cancer cells. J Vis Exp. 2015; e52727. https://doi.org/10.3791/52727-v PMid:26067809 PMCid:PMC4542786
37. Yang T, Zhai H, Yan R, Zhou Z, Gao L, Wang L. lncRNA CCAT1 promotes cell proliferation, migration, and invasion by down-regulation of miR-143 in FTC-133 thyroid carcinoma cell line. Braz J Med Biol Res. 2018;51. https://doi.org/10.1590/1414-431x20187046 PMid:29791590 PMCid:PMC6002139
38. Vuik FE, Nieuwenburg SA, Bardou M, Lansdorp-Vogelaar I, Dinis-Ribeiro M, Bento MJ, Zadnik V, Pellisé M, Esteban L, Kaminski MF. Increasing incidence of colorectal cancer in young adults in Europe over the last 25 years. Gut. 2019; 68:1820-6. https://doi.org/10.1136/gutjnl-2018-317592 PMid:31097539 PMCid:PMC6839794
39. Levin B, Lieberman DA, McFarland B, Andrews KS, Brooks D, Bond J, Dash C, Giardiello FM, Glick S, Johnson D. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. Gastroenterology. 2008; 134:1570-95. https://doi.org/10.1053/j.gastro.2008.02.002 PMid:18384785
40. Chabner B, Longo D. Clinical strategies for cancer treatment: the role of drugs. In: Cancer Chemotherapy and Biotherapy: Principles and Practice. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
41. Aslam MS, Naveed S, Ahmed A, Abbas Z, Gull I, Athar MA. Side effects of chemotherapy in cancer patients and evaluation of patients' opinion about starvation-based differential chemotherapy. J Cancer Ther. 2014;2014. https://doi.org/10.4236/jct.2014.58089
42. Safarzadeh E, Shotorbani SS, Baradaran B. Herbal medicine as inducers of apoptosis in cancer treatment. Adv Pharm Bull. 2014; 4:421.
43. Smidt E, Meissl K. The applicability of Fourier transforms infrared (FT-IR) spectroscopy in waste management. Waste Manag. 2007; 27:268-76. https://doi.org/10.1016/j.wasman.2006.01.016 PMid:16530397
44. Skowyra M, Calvo MI, Gallego Iradi MG, Azman NABM, Almajano Pablos MP. Characterization of phytochemicals in petals of different colours from Viola·wittrockiana Gams and their correlation with antioxidant activity. J Agric Sci. 2014; 6:93-105. https://doi.org/10.5539/jas.v6n9p93
45. Selvakumar P, Badgeley A, Murphy P, Anwar H, Sharma U, Lawrence K, Lakshmikuttyamma A. Flavonoids and other polyphenols act as epigenetic modifiers in breast cancer. Nutrients. 2020; 12:761. https://doi.org/10.3390/nu12030761 PMid:32183060 PMCid:PMC7146477
46. Mahboubi M, Kashani LMT. A narrative study about the role of Viola odorata as traditional medicinal plant in management of respiratory problems. Adv Integr Med. 2018; 5:112-8. https://doi.org/10.1016/j.aimed.2017.12.003
47. Sarker U, Oba S. Polyphenol and flavonoid profiles and radical scavenging activity in leafy vegetable Amaranthus gangeticus. BMC Plant Biol. 2020; 20:1-12. https://doi.org/10.1186/s12870-020-02700-0PMid:33138787 PMCid:PMC7607633
48. Sato Y, Itagaki S, Kurokawa T, Ogura J, Kobayashi M, Hirano T, Sugawara M, Iseki K. In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. Int J Pharm. 2011; 403:136-8. https://doi.org/10.1016/j.ijpharm.2010.09.035 PMid:20933071

49. Silva MM, Santos MR, Caro| o G, Rocha R, Justino G, Mira L. Structure-antioxidant activity relationships of flavonoids: a re-examination. Free Radic Res. 2002; 36:1219-27. https://doi.org/10.1080/198-1071576021000016472 PMid:12592674
50. Herrmann A, Svang∑rd E, Claeson P, Gullbo J, Bohlin L, G\ransson U. Key role of glutamic acid for the cytotoxic activity of the cyclotide cycloviolacin O2. Cell Mol Life Sci. 2006; 63:235-45. https://doi.org/10.1007/s00018-005-5486-4 PMid:16389447 PMCid:PMC11136400
51. Silva MT. Secondary necrosis: the natural outcome of the complete apoptotic program. FEBS Lett. 2010; 584:4491-9. https://doi.org/10.1016/j.febslet.2010.10.046 PMid:20974143
52. El-Kott AF, Abd-El-Karim M, Khalifa HS, Morsy K, Ibrahim EH, Bin-Jumah M, Abdel-Daim MM, Aleya L. Kaempferol protects against cadmium chloride-induced hippocampal damage and memory deficits by activation of silent information regulator 1 and inhibition of poly (ADP-Ribose) polymerase-1. Sci Total Environ. 2020; 728:138832. https://doi.org/10.1016/j.scitotenv.2020.138832 PMid:32353801
53. Yu D, Liu C, Guo L. Mitochondrial metabolism and cancer metastasis. Ann Transl Med. 2020;8. https://doi.org/10.21037/atm.2020.03.42 PMid:32793748 PMCid:PMC7396750
54. Zeinoddini S, Nabiuni M, Jalali H. The synergistic cytotoxic effects of doxorubicin and Viola odorata extract on human breast cancer cell line T47-D. J Cancer Res Ther. 2019; 15:1073. https://doi.org/10.4103/jcrt.JCRT_990_17 PMid:31603113
55. Rauf A, Imran M, Khan IA, ur-Rehman M, Gilani SA, Mehmood Z, Mubarak MS. Anti-cancer potential of quercetin: A comprehensive review. Phytother Res. 2018; 32:2109-30. https://doi.org/10.1002/ptr.6155 PMid:30039547
56. McSherry EA, Brennan K, Hudson L, Hill AD, Hopkins AM. Breast cancer cell migration is regulated through junctional adhesion molecule-A-mediated activation of Rap1 GTPase. Breast Cancer Res. 2011; 13:1-14. https://doi.org/10.1186/bcr2853 PMid:21429211 PMCid:PMC3219194
57. Sun DW, Mao L, Zhang J, Jiang LH, Li J, Wu Y, Ji H, Chen W, Wang J, Ma R. MiR-139-5p inhibits the biological function of breast cancer cells by targeting Notch1 and mediates chemosensitivity to docetaxel. Biochem Biophys Res Commun. 2015; 465:702-13. https://doi.org/10.1016/j.bbrc.2015.08.053 PMid:26299922
58. Tuasha N, Seifu D, Gadisa E, Petros B, Oredsson S. Solvent fractions of selected Ethiopian medicinal plants used in traditional breast cancer treatment inhibit cancer stem cells in a breast cancer cell line. BMC Complement Med Ther. 2020; 20:1-9 https://doi.org/10.1186/s12906-020-03154-5 PMid:33238963 PMCid:PMC7687706