ANTIOXIDANT AND CYTOTOXIC EFFECTS OF ESSENTIAL OILS EXTRACTED FROM *Viola odorata* L. CULTIVATED IN IRAQ

Zainab O. Salman **Shaymaa I. K. Al-juboori** **Bushra M.J. Alwash**
Lecturer **Assist. Prof.** **Prof.**
Dept. of Bio., Coll. Of Sci. for Women, Univ. of Baghdad, Baghdad, Iraq
Zainab19831210@ csw.uobaghdad.edu.iq shamaa.i@csw.uobaghdad.edu.iq
bushraalwash1966@gmail.com

ABSTRACT

This study was aimed to determine the composition of essential oil contained in *Viola odorata* flowers and leaves. As well as assessing the cytotoxic effects of this oil on cancer cells and its antioxidant properties. A GC-Mass analysis was conducted to reveal essential oil components in flowers and leaves. For testing the antioxidant capacity of flowers and leaves oil, DPPH (1,1 Dyphenyl-2-picrylhydrazyl), resazurin dye, and hydroxyl were used. An in vitro study was conducted using lung cancer (A549) and breast normal (MCF-10) cell lines with concentrations of 25, 50, 100, 200, and 300 µl/ml of *V. odorata L.* essential oil. According to the results, essential oils derived from flowers and leaves contain different components in terms of quality and quantity. At a concentration of 300 µl/ml, the results showed that flowers' essential oil was highly antioxidant (98.16 %, 92.47%, 94.00 %) when combined with DPPH, resazurin dye, and hydroxyl, respectively. There was variation in cytotoxic effects on cancer cells based on oil concentrations and sources (flowers and leaves). In conclusion, the A549 cell line was significantly affected by flower oil than by leaves oil. The highest effect of flower oil was observed at a concentration of 300 µl/ml.

Keywords: *Viola odorata L.*, lung cancer, essential oils, antioxidant, cytotoxic activity.

التأثيرات المضادة للأكسدة والسامة للخلايا لمستخلص الزيوت العطرية لنبات البنفسج العطري *Viola odorata L.* المزروع في العراق

زينب عمران سلمان شيماء اسماعيل كاظم بشرى محمد جابر
مدرس استاذ مساعد استاذ
قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق

المستخلص

الغرض من هذه الدراسة هو تحديد تركيبة الزيت العطري الموجود في زهور وأوراق نبات البنفسج العطري *V. odorata*. وكذلك تقييم التأثيرات السامة للخلايا لهذا الزيت على الخلايا السرطانية وخصائصه المضادة للأكسدة. تم إجراء تحليل GC–Mass للكشف عن مكونات الزيت العطري في الزهور والأوراق. لاختبار القدرة المضادة للأكسدة لزيت الزهور والأوراق، تم استخدام DPPH (1،1 ديفينيل –2 بيكريل هيدرازيل)، وصبغة الريسازورين، والهيدروكسيل. أجريت دراسة في المختبر باستخدام خطوط خلايا سرطان الرئة (A549) وخلايا الثدي الطبيعية (MCF–10) بتركيزات 25 و50 و100 و200 و300 ميكرولتر/مل من زيت *V. odorata L.* العطري. ووفقا للنتائج، فإن الزيوت العطرية المشتقة من الزهور والأوراق تحتوي على مكونات مختلفة من حيث الجودة والكمية. أظهرت النتائج أن الزيت العطري المستخلص من الزهور وبتركيز 300 ميكرولتر/مل، كان مضادًا للأكسدة بدرجة عالية (98.16%، 92.47%، 94.00%) عند استخدامه معDPPH ، وصبغة الريسازورين، والهيدروكسيل، على التوالي. كان هناك تباين في التأثيرات السمية على الخلايا السرطانية بناءً على تراكيز ومصادر الزيت (الزهور والأوراق). نستنتج من ذلك أن الخط الخلوي A549 تأثر معنوياً بزيت الزهور مقارنة بزيت الأوراق، ولوحظ أعلى تأثير لزيت الزهورعند تركيز 300 ميكرولتر/مل.

الكلمات المفتاحية: *Viola odorata L.*، سرطان الرئة، الزيوت العطرية، مضادات الأكسدة، النشاط السام للخلايا

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INTRODUCTION

There are a number of plant extracts that contain useful components for pharmacotherapy (23, 24). *Viola odorata* L. (sweet violet), for example, contains anticancer components including phenol, cyclotide, and anthocyanins. It is a fragrant member of the viola family that grows wild in Europe as well as Asia. It is a tough little herbaceous perennial. This flower has been given a number of names, including sweet violet and English violet (1-11).Studies on the phytochemistry of different parts of *V. odorata* revealed the presence of coumarins, caffeic acid, methyl salicylate, flavonoids (*Quercetin, kaempferol*), glycosides (Rutin) (12), as well as terpenoids (stigma sterol) (13). *V. odorata* has been used in medicine for a long time. Among the many medicinal uses of *V. odorata* are expectorants, antipyretics, antibacterial, diuretics, and laxatives. The vapors are used to treat asthma, coughing, and bronchitis(11, 14). Coumarins are abundant in *V. odorata* (11). A good deal of pharmacological ingredients can be found in herbal medicine, which can be used to treat cancer and tumors (15, 18) . The active constituents of this plant include cyclotides (16), volatile oils, violins, odorutins, rutins, syanyns, bright pigments, methyl salicylate glycosides, and anthocyanins (17). Anthocyanins, phenols, and cyclotides are some of the most important metabolites with antioxidant and anticancer properties (1, 3, 32). It has traditionally been used to treat anxiety, insomnia, hypertension, diuretic, and laxative disorders (11, 21). Several studies have demonstrated that the hydroalcoholic extract of *V. tricolor* is effective against breast and neuroblastoma tumors (19). The herb *V. odorata* is known for its anti-inflammatory and anti-rheumatic properties, anti-hypertensive and antioxidant characteristics, and anti-cancer properties (9, 20) . It has been reported that the alcoholic extract of *V. odorata* leaves has anti-metastatic properties in traditional medicine. Saponin, salicylic acid derivatives, glycosides such as vioacercitin, alkaloids such as violins, anthocyanidins, and cyclotides such as cycloviolacin are the active components of *V. odorata*. These compounds have also been shown to have anti-cancer properties in various research studies (20, 29). Traditionally, *V. odorata* has been considered a medicinal herb, used to treat anxiety, lower blood pressure, bronchitis, kidney and liver disorders as well as relieve cancer pain. *V. odorata* has also been reported to possess anti-inflammatory, antipyretic, antioxidant, and antibacterial properties (22). In cancer treatment, fresh leaves of *V. odorata* have been used both internally and externally. Cancer of the throat or tongue can be treated with decoctions, poultices, infusions, syrups from petals, or liquid extracts. Further, some Violaceae compounds (cyclotides and flavonoids) showed significant anticancer and antioxidant properties (1). In general, 15% of patients with lung cancer are diagnosed with small-cell lung cancer (SCLC). Smoking is the leading cause of SCLC in almost all cases (24). There is a rapid progression of SCLC and a tendency for widespread metastasis(25). Over the past several decades, platinum-based chemotherapy regimens have been the standard of care for patients with ES-SCLC. The median survival for patients with ES-SCLC rarely exceeds one year, despite a good initial response to treatment. It is estimated that only 10-20% of patients with ES-SCLC will survive beyond two years after their initial diagnosis(26). This study was aimed to determine the essential oil composition of the flowers and leaves of *Viola odorata.* This oil was also evaluated for its antioxidant and cytotoxic effects on cancer cells.

MATERIALS AND METHODS

The Iraqi National Herbarium authenticated the flowers and leaves collected from fields in Baghdad, Iraq, in May 2022. A series of steps were taken to clean, dry, grind, and store them at 4°C. The chemical materials and solvents used in this study were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, USA). A Clevenger apparatus was used to isolate essential oils from 50gm of flowers or leaves. The oil obtained was stored in a dark bottle for further use.

Qualitative and quantitative analysis

In order to determine the quality and quantity of essential oil components, GC-MS analysis was performed on GC-MS QP-2010 (Shimadzu Instruments, Japan). InertCap Pure Wax capillary column was used (30 m x 0.25 mm x 0.25 µm film thickness) with helium as

carrier gas at a flow rate of X ml/min. The source was operated in positive ionization mode (electron impact energy: 70eV) and the detection was performed in full-scan mode. The inlet and the transfer line temperatures were maintained at X°C, while the ion source was kept at X°C. Samples were injected in split or splitless mode (2:1) and separated using a temperature gradient program as follows: XºC for Xmin, to XºC at XºC/min and then maintained at XºC for Xmins; then to XºC at XºC/min and maintained at XºC for further X mins. GC-MS spectra were evaluated by Postrun software and searched in the National Institute of Standards and Technology (NIST) MS Search V2.0 browsers.

Antioxidant Activity: 1,1 Dyphenyl-2 picrylhydrazyl (DPPH), resazurin dye, and hydroxyl were used to quantify the scavenging ability of free radicals in the essential oil of *V. odorata* flowers and leaves. In equal volumes of DPPH, resazurin dye, and hydroxyl, oil concentrations of 25, 50, 100, 200, and 300 µl/ml were mixed with DPPH, resazurin dye and hydroxyl. After 15 minutes, the absorbance was measured at a wavelength of 600 nm by a spectrophotometer against a blank solution containing only DW (17, 18). In this study, ascorbic acid was used as a standard (positive control). In order to calculate the scavenging activity, the following formula was used:

$$\textit{Scavenging activity}\ \% = \frac{\textit{A600 of control} - \textit{A600 of sample}}{\textit{A600 control}}\ x\ 100$$

Cytotoxic activity : Cell lines used in this study were provided by the Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR). The work was conducted using two types of cell lines: lung cancer (A549 cells) and breast cancer (MCF-10 cells) as normal cellular lines. Approximately 500 µl/ml of essential oil from *V. adorata* flowers were dissolved in 500 µl/ml of solution (media and dimethyl sulfoxide). During the log phase of growth, cell cultures were exposed to a range of essential oil concentrations in microtitration plates (96 wells) and their effects were evaluated following recovery. Cell lines were exposed for 72 hours in a microtitration plate under completely sterile conditions. Each cell line was tested with different concentrations of essential oil 25, 50, 100, 200, and 300 µl/ml, with three replicates for each concentration(27).

Statistical analysis

Statistical Analysis System - SAS (2012) was used to analyze the effects of different factors on study parameters. In this study, the least significant difference (LSD) test was used to determine whether there was a significant difference between means(28).

RESULTS AND DISSCUSION

Essential oil components qualitative and quantitative analysis : Based on GC-Mass analysis, the essential oils derived from *V.odorata* flowers and leaves contain different components in terms of quality and quantity, as shows in Tables (1) and (2). There were (21 compounds) identified by the analysis, accounting for (25.64 %) of the oil, with dipropylene glycol and (24.90 %) squalene being the two main components of the flowers. Eighteen compounds were identified in the leaves, with cyclohexasiloxane and dipropylene glycol constituting (56.03%) and (11.22%) respectively. In addition, figures (1) and (2) depict the different components of the essential oils extracted from *V.odorata* flowers as well as the leaves.

Table 1. Essential oil components concentrations' of *V.odorata* flowers

N	Name of compound	%
1	Ankilostin	1.97
2	Diacetone alcohol	1.77
3	Dipropylene glycol	25.64
4	Dimethoxypropane	6.28
5	Dihydromyrcenol	2.54
6	Phenethyl alcohol	1.40
7	Benzyl benzoate	5.81
8	Terpineol	0.75
9	Borneol, acetate	4.4
10	Butylcyclohexyl acetate	4.42
12	Verdyl acetate	4.52
13	benzyl acetate	1.46
14	Cinnamaldehyde	1.97
15	Methyl 11-octadecenoate	2.55
16	Ethyl 9-octadecenoate	8.32
17	Oleic Acid	0.47
18	Squalene	24.90
19	Malonic acid	1.32
20	Vitamin E	0.78
21	Ergosterol	1.28

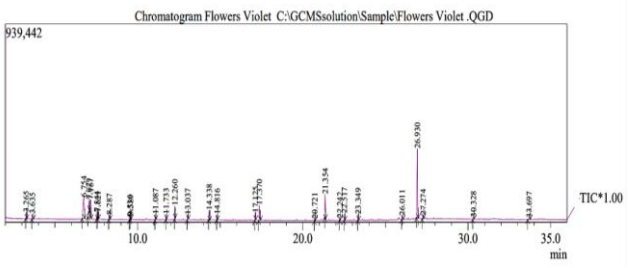

Figure 1. GC-mass analysis of essential oil of V.adorata flowers

Table 2. Essential oil components concentrations' of *V.odorata* leaves

N	Name of compound	%
1	Ankilostin	6.26
2	Diacetone alcohol	4.84
3	Dipropylene glycol	11.22
4	Phenylethyl alcohol	0.36
5	terpineol	0.25
6	Borneol acetate	0.46
7	Butylcyclohexyl acetate	1.38
8	Verdyl acetate	1.75
9	benzyl acetate	0.58
10	Hexadecamethyl-cyclooctasioxane	2.03
12	Benzyle benzoate	1.42
13	cyclohexasiloxane	56.03
14	Palmatic acid	1.31
15	Ethyl 9-octadecenoate	2.38
16	Oleic Acid	0.32
17	Methyl 11-octadecenoate	1.45
18	Squalene	6.35

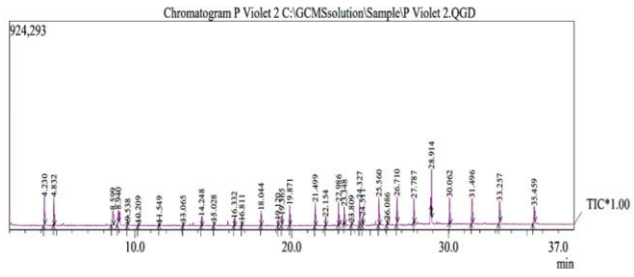

Figure 2. GC-mass analysis of essential oil of V.adorata leaves

In a similar study in Kashan, central Iran, essential oils were extracted by hydro distillation-solvent extraction and analyzed using GC-MS. Twenty-five compounds were identified, including butyl-2-ethylhexylphthalate and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranon. In another study, it was found that *V. odorata L*. essential oil contains high levels of monoterpenes and sesquiterpenes. An analysis of the chemical composition of *V. odorata L*. essential oil revealed that 63 components were identified by GC-MS. These components represent (83.05) percent of the total oil. (30). In addition, a review article of species of the genus Viola identified chemical constituents (as salicylic acid methyl ester), as well as(aodoratine, 2,2,6,6-tetramethyl-4-piperidinone), (violacin A), (vitripeptide A), (vodopeptide M and N), (2-nitroproprionic acid), (mucilage)(vitamin C), (cyclotides), and (anthocyanins). The highest number of isolated compounds were identified in *V. odorata*, while the lowest number was identified in *V. thianschanica*. The predominant isolated compounds in the various species were (sesquiterpenes and aliphatic) compounds, but not in the species of *V. thianschanica*. It is likely that these constituents are a result of the different location and/or time at which the plants were collected(31).

Antioxidant activity *in vitro*

An assessment of antioxidant activity was conducted, and the results were expressed as a percentage of inhibition. We then compared the antioxidant activity of the extract with that of ascorbic acid, which was used as a standard. As shown in (Table 3,4,5) flowers oil exhibits antioxidative activity at 300 µl /ml. By using DPPH, resazurin dye, and hydroxyl separately, this oil demonstrated high antioxidant activity (98.16 %, 92.47%, 94.00 %) respectively. Similar results were observed by (24). The researchers found that the survival rate of astrocytes from human GBM tumors decreased when treated with the hydroalcoholic extract of *V. odorata* (10 - 500 µg/mL) over time and in a dose-dependent manner.

Table 3. Antioxidant activity % against DPPH free radical of essential oil of flower of *V.odorata* plant.

Concentration (mg/ml)	Mean ± SE			LSD value
	Flower oil	Leaf oil	Ascorbic acid	
25	79.16 ± 0.12 E a	50.57 ± 0.07 E c	75.06 ± 0.03 D b	0.288 **
50	85.52 ± 0.02 D a	64.20 ± 0.06 D b	81.26 ± 0.63 C a	1.271 **
100	92.97 ± 0.05 C a	78.10 ± 0.04 C a	86.31 ± 0.06 B a	0.182 **
200	95.09 ± 0.01 B a	82.71 ± 0.06 B b	95.56 ± 0.04 A a	0.151 **
300	98.16 ± 0.08 A a	90.91 ± 0.09 A c	95.73 ± 0.03 A b	0.262 **
LSD value	0.220 **	0.220 **	0.901 **	---

** (P<0.01).

Means having with the different big letters in same column and small litters in same row differed significantly

Table 4. Antioxidant activity % against Hydroxyl free radical of essential oil of flower of *V.odorata* plant

Concentration (mg/ml)	Mean ± SE		LSD value
	Flower oil	Ascorbic acid	
25	46.67 ± 1.764 D b	74.03 ± 1.323 D a	5.922 **
50	65.43 ± 2.067 C b	80.20 ± 1.432 C a	6.025 **
100	73.67 ± 1.202 B b	84.36 ± 1.403 BC a	5.869 **
200	84.77 ± 1.534 A b	90.06 ± 1.510 AB a	5.021 **
300	92.47± 1.444 A a	92.00 ± 1.528 A a	3.261 NS
LSD value	6.027 **	5.955 **	---

Means with different big letters in the same row and small letters in the same column are significantly different. ** (P≤0.01).

Table 5. Antioxidant activity % against Resazurin free radical of essential oil of flower of *V.odorata* plant.

Concentration (mg/ml)	Mean ± SE		LSD value
	Flower oil	Ascorbic acid	
25	52.33 ± 2.828 D b	74.03 ± 1.323 C a	6.442 **
50	67.67 ± 2.333 C b	80.20 ± 1.432 BC a	6.075 **
100	79.00 ± 2.517 B a	84.36 ± 1.403 AB a	5.315 NS
200	90.67 ± 1.202 A a	90.06 ± 1.510 A a	2.084 NS
300	94.00 ± 1.528 A a	92.00 ± 1.528 A a	3.016 NS
LSD value	7.692 **	6.871 **	---

Means with different big letters in the same row and small letters in the same column are significantly different. ** (P≤0.01)

Our finding is compatible with a study published by Aslam and colleagues in 2020 described the significant radical scavenging activity of ethanol extract (EC50 160 µg mL−1) and ferric reducing ability (16.9 eq equivalent mol Fe2+/g sample) (8). The natural plant compounds stimulate antioxidant activity, decrease the production of pro-inflammatory cytokines, lower the amount of nitrite produced in the body, reduce harmful oxidants, alleviate nerve pain, reduce harmful oxidants, decrease nitrite production, and activate neuroprotective mechanisms within the nervous system, among many others. Numerous studies have demonstrated that *V. odorata L.* leaf extract and some of its molecular components are capable of protecting against toxic insults. Indirectly, this occurs through antioxidant properties or directly through neuroprotective mechanisms (33). This genus contains several plant species with pharmacologically significant pharmacological activities, including neuroprotection, immunomodulation, anticancer, antihypertensive, antidyslipidemic, analgesic, antipyretic, diuretic, anti-inflammatory, antihelmintic, and antioxidant properties (31).

Cytotoxic activity: During our in vitro experiment, we used lung cancer (A549) and normal breast (MCF-10) cell lines with concentrations of (25, 50, 100, 200, and 300) µl/ml of *V. odorata L.* leaf and flower essential oils. The results indicated that the cytotoxic effects of oils on cancer cells vary according to the concentration of the oil and the source of the oil (flowers and leaves). Both Table 6 and Figure 4 demonstrate the cytotoxic activity of essential oil of flowers on A549 and MCF10 cell lines (88.57 % and 13.10 %), respectively. There was a significant difference between the effects of flower oil and leaves oil on the A549 cell line, and the highest effect of flower oil was observed at a concentration of 300 µl/ml.

Table 6. Cytotoxic activity % of essential oil of flower on A549 and MCF10 cell lines.

Concentration (µg/ml)	Cell line		LSD value
	549/Lung cancerA	MCF10/breast cell	
25	13.67 ±1.525 E a	2.167 ±0.4410 D b	3.167 **
50	29.43 ±2.890 D a	4.267 ±0.3712 CD b	4.075 **
100	53.00 ±2.309 C a	7.300 ±0.9074 BC b	4.182 **
200	78.27 ±1899 B a	10.50 ±0.8660 AB b	4.244 **
300	88.57 ±2.413 A a	13.10 ±1.222 A b	5.902 **
LSD value	6.034 **	3.913 **	---

Means with different big letters in the same row and small letters in the same column are significantly different. ** (P≤0.01).

Further, (Figure 3) illustrates the morphological changes in A549 cells and MCF-10 cells following treatment with 300 µl/ml essential oil of *V.odorata* plant flowers after 72 hours at a magnification power of 400x compared to control untreated cells.

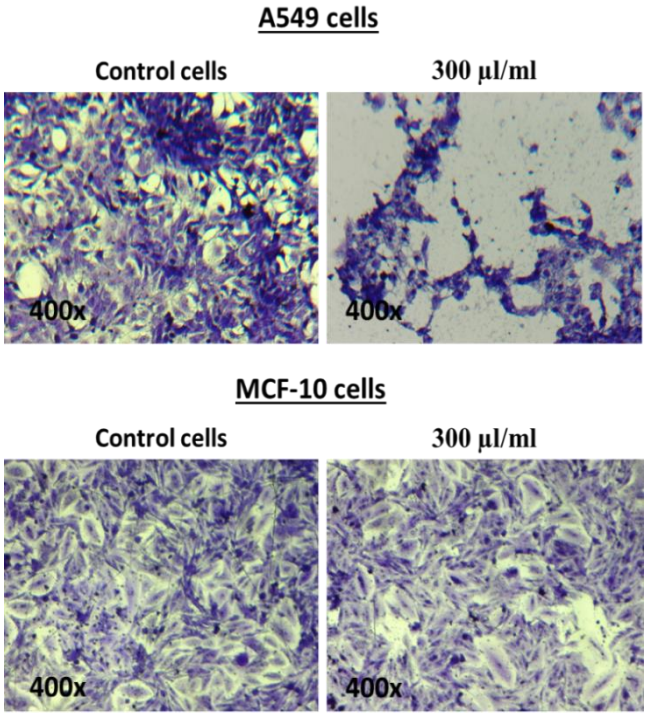

Figure 3. Morphological changes in A549 cells and in MCF-10 cells after treated with 300 µl/ml essential oil of *V.odorata* plant flowers in comparison with control untreated cell lines after 72 hours. Magnification power 400x.

This result agrees with (33) who found that *V. odorata* extract caused apoptosis in MCF7, SKBR3 and their derived mammospheres, but did not affect MCF10A. Further, this extract exhibited anti-migratory, anti-invasion, and anti-colony formation activity in MCF7, SKBR3, and their associated mammospheres, which was significantly higher in mammospheres induced by MCF7 and SKBR3. The extract also reduced the size and volume of tumors induced by MCF7, SKBR3, and their derived mammospheres in chicken embryos. Another study showed that (50.98-nm) *V. odorata* Essential Oil Nanoemulsion (VEO-NE) significantly reduced viability and induced apoptosis in A2780 ovarian cancer cells (34). Furthermore, an investigation demonstrating the anticancer activity of some medicinal plants identified *V.odorata* as one of the therapeutic plants with anticancer properties(35). According to several studies, *V. odorata* essential oil has antioxidant, antimicrobial, antiproliferative, and anticancer properties, including breast cancer, human glioblastoma, and others(34-36). *V.odorata* comprises more than 500 species throughout the world, making it the largest genus of the Violaceae family. A thorough review of the literature revealed that Viola species are valuable nutritional and medicinal plants used in the ethnomedical treatment of noncommunicable diseases (NCDs) such as diabetes, asthma, lung disease, and fatigue. Furthermore, several plants in this genus have been scientifically validated as having medicinal properties.

The beneficial properties of these agents include neuroprotective,immunomodulatory, anticancer, antihypertensive, antidyslipidemic, diuretic, anti-inflammatory, antihelminthic, and antioxidant properties. Over the past few decades, a number of natural products have been isolated and identified, including flavonoids, terpenoids and phenylpropanoids. These compounds have a wide range of potential therapeutic applications, and may be a promising source of new drugs(31)

The flowers and leaves of the Viola plant are used in alternative medicine to treat respiratory ailments such as congestion, coughing, and sore throats. Decoctions made from the root (dry herb) are used as laxatives. It has been used for centuries for the treatment of headaches, body pains, and sedation, and more recent research has detected the presence of a glycoside of salicylic acid (natural aspirin) in the plant(30).

As a conclusion: the extract of *V. odorata* has been shown to possess anti-cancer properties in A549 cells. The anti-cancer activity of this extract was significantly greater in A549 cell lines than in MCF-10 cell lines. There is evidence that *V. odorata* extract primarily targets cancerous cells, not normal cells. It acts in a cell-dependent manner. According to the results of this study, *V. odorata* extract has a potential role in cancer therapy due to its mechanism of action.

REFERENCES

1.Al-Khafaji, A. M. H. H. 2019. Stimulation growth, yield, and accumulation of antioxidant

compounds of onion hybrids by colored shades of poly ethylene covers. Iraqi Journal of Agricultural Sciences, 50(6): 1580-1587. https://doi.org/10.36103/ijas.v50i6.847

2.Akhbari, M., H. Batooli and F. J. Kashi. 2012. Composition of essential oil and biological activity of extracts of *Viola odorata* L. from central Iran. Natural product research, 26(9):802-9.
doi: 10.1080/14786419.2011.55801

3.Albandary, N. 2023. Phenolic compounds content, antioxidant, antibacterial and antifungal activities of red onions skin. Iraqi Journal of Agricultural Sciences, 54(4):1050-1057. https://doi.org/10.36103/ijas.v54i4.1794

4.Ali, Z. A., I. Saleh and W. M. Alani. 2023. Detection of Coumarin Derivatives of *Viola odorata* Cultivated in Iraq. Journal of Pharmacy and Bioallied Sciences, 15(Suppl 2):S948-S51. DOI: 10.4103/jpbs.jpbs_270_23

5.Alipanah H., M. R. Bigdeli and M.A. Esmaeili. 2018. Inhibitory effect of *Viola odorata* extract on tumor growth and metastasis in 4T1 breast cancer model. Iranian journal of pharmaceutical research: IJPR.,17(1):276.

6.Arora, R., S. Sawney, V. Saini, C. Steffi, M. Tiwari and D. Saluja. 2016. Esculetin induces antiproliferative and apoptotic response in pancreatic cancer cells by directly binding to KEAP1. Molecular cancer, 15:1-15. DOI 10.1186/s12943-016-0550-2

7. Albandary N. A. 2023. Testing and evaluation of bioactive compounds in soybean. Iraqi Journal of Agricultural Sciences, 54(1): 85-92.
https://doi.org/10.36103/ijas.v54i1.1678

8.Aslam, L., R. Kaur, N. Kapoor and R. Mahajan. 2020. Phytochemical composition and antioxidant activities of leaf extracts of *Viola odorata* from Kishtwar, Jammu and Kashmir. Journal of Herbs, Spices & Medicinal Plants, 26(1):77-88.
DOI: 10.1080/10496475.2019.1677839

9., Anwar, F, U Saleem, S. Hira, , M. A. Shah, S. Bashir, R. S. Baty, R. H. Badr, R. Blundell, G. E. Batiha, and B. Ahmad. 2021. Pharmacological screening of *Viola odorata* L. for memory-enhancing effect via modulation of oxidative stress and inflammatory biomarkers. Frontiers in Pharmacology, 12, 664832.
https://doi.org/10.3389/fphar.2021.664832

10.Baek, J. M., S. H. Park, Y. H. Cheon, S. J. Ahn, M. S. Lee, J. Oh and *et al*. 2015. Esculetin attenuates receptor activator of nuclear factor kappa-B ligand-mediated osteoclast differentiation through c-Fos/nuclear factor of activated T-cells c1 signaling pathway. Biochemical and Biophysical Research Communications, 461(2):334-41.
DOI: 10.1016/j.bbrc.2015.04.034.

11.Batiha, G. E., H. Y. Lukman, H. M. Shaheen, L. Wasef, A. A. Hafiz, C. A. Conte-Junior and *et al*. 2023. A Systematic Review of Phytochemistry, Nutritional Composition, and Pharmacologic Application of Species of the Genus Viola in Noncommunicable Diseases (NCDs). Evidence-Based Complementary and Alternative Medicine, 2023: 5406039.
DOI: 10.1155/2023/5406039

12.Bray, F., J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre and A. Jemal. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal For Clinicians, 68(6):394-424.
DOI: 10.3322/caac.21492

13.Cary, N. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

14.Dhiman, S., S. Singla, I. Kumar, P. Palia, P. Kumar and S. Goyal. 2023. Protection of *Viola odorata* L. against neurodegenerative diseases: potential of the extract and major phytoconstituents. Clinical Complementary Medicine and Pharmacology,100105.
https://doi.org/10.1016/j.ccmp.2023.100105

15.Doğan, M., F. S. Mohammed, İ. Uysal, K. Mencik, K. Eylem, M. Pehlivan and *et al*. 2023. Total antioxidant status, antimicrobial and antiproliferative potentials of *Viola odorata* (Fragrant Violet). Journal of Faculty of Pharmacy of Ankara University, 47(3): 784-791. DOI: 10.33483/jfpau.1161440

16.Ebrahimzadeh, M. A., S. M. Nabavi, S. F. Nabavi, F. Bahramian and A. R. Bekhradnia. 2010. Antioxidant and free radical scavenging activity of H. officinalis L. var. angustifolius, *V. odorata*, *B. hyrcana* and *C. speciosum*. Pak

J Pharm Sci, 23(1):29-34.
https://pubmed.ncbi.nlm.nih.gov/20067863
17.Gautam, S. S. and K. S. Navneet. 2017. Current aspects on phytochemistry and bioactive constituents of *viola odorata* L. Indian J Biotechnol Pharmaceut Res, 5:1-6. https://www.researchgate.
18.Hamad, S., Z. Salman and B. Alwash. 2021. Assesment of antioxidant and cytotoxic activiy of essential oil extracted from *Lavandula angustifolia* callus leaves. Iraqi Journal of Agricultural Sciences, 52(6):1549-1554. https://doi.org/10.36103/ijas.v52i6.1496
19.Hammami, I., N. Kamoun and A. Rebai. 2011. Biocontrol of Botrytis cinerea with essential oil and methanol extract of *Viola odorata* L. flowers. Arch Appl Sci Res, 3(5):44-51.
http://www.scholarsresearchlibrary.com.
20. Hossein, B. Akhbari, M, and F J Kashi. 2023. Composition of essential oil and biological activity of extracts of Viola odorata L. from central Iran. Natural product research, 26(9), 802-809.
21. Jafari, F. Z. Feyzabadi, S. H. Kamali, H. Ashayeri, S B Aval, M M Esfahani, and O Sadeghpour. 2014. Efficacy of Viola odorata in treatment of chronic insomnia. Iranian Red Crescent Medical Journal, 16(12): :e17511
22.Jasim, S. F., N. N. Baqer and E. Alraheem. 2018. Detection of phytochemical constituent in flowers of *Viola odorata* by gas chromatography-mass spectrometry. Asian Journal of Pharmaceutical and Clinical Research, 11(5):262-9.
DOI:https://doi.org/10.22159/ajpcr.2018.v11i5.24288
23.Lazeeza, S. 2021. Antioxidant activity of pomegranate. Iraqi Journal of Agricultural Sciences, 52(1):196-203.
https://doi.org/10.36103/ijas.v52i1.1251
24. Machado, Y., W. Murillo-Arango and L. Hennessey-Ramos. 2022. Evaluation of peel extract of mangosteen as a dye natural and antioxidant and its use as an additive in a fruit beverage. Iraqi Journal of Agricultural Sciences, 53(4):857-866.
https://doi.org/10.36103/ijas.v53i4.1598
25.Mak, D. W., S. Li and A. Minchom. 2019. Challenging the recalcitrant disease developing molecularly driven treatments for small cell lung cancer. European Journal of Cancer, 119:132-50.
DOI: 10.1016/j.ejca.2019.04.037
26.Mazimba, O. 2017. Umbelliferone: Sources, chemistry and bioactivities review. Bulletin of Faculty of Pharmacy, Cairo University, 55(2):223-32.
https://doi.org/10.1016/j.bfopcu.2017.05.001
27.Miller, K. D., M. Fidler-Benaoudia, T. H. Keegan, H. S. Hipp, A. Jemal and R. L. Siegel. 2020. Cancer statistics for adolescents and young adults.CA: A Cancer Journal for Clinicians, 70(6):443-59.
DOI: 10.3322/caac.21637
28.Motavasselian, M., R. Salari, Z. Feyzabadi, M. R. Joharchi and S. M. Ghazanfari. 2022. A review of the therapeutic effects of *Viola odorata* plant in traditional iranian medicine and modern medicine. Complementary Medicine Journal, 12(2):118-25.
DOI: 10.32598/CMJA.12.2.1133.2
29. Moosa, T, O Ané, N Motala, G Kamatou, A Viljoen, and S van Vuuren. 2023. A Commercially available Viola odorata oil, chemical variability and antimicrobial activity. Molecules, 28(4), 1676.
https://doi.org/10.3390/molecules28041676
30.Sahib Abed, H., P. Zarearki, V. Khojasteh, E. Karimi, K. Shahrokhabadi and M. Rastegar Moghaddam Poorbagher. 2024. Inhibition the growth of human ovarian cancer cells (A2780) via cell proliferation and zngiogenesis by *Viola odorata* essential oil nanoemulsion. Waste and Biomass Valorization,1-10.
DOI:10.1007/s12649-023-02314-1
31.Sultana, S., Z. A. Mahdi, N. S. K. Al-Khafaji, H. O. M. Al-Dahmoshi, A. Rashid, M. Akram and *et al*. 2023. Anticancer activity of some medicinal plants: minireview. Journal of Medical Research and Health Sciences, 6(5):2527−2538.
DOI: https://doi.org/10.52845/JMRHS/2023-6-5-1
32.Yousif, A. and W. Hassan. 2023. HPLC analysis and antifungal activity of some plant extracts against decay apple fruits. Iraqi Journal of Agricultural Sciences, 54(1):291-302.
https://doi.org/10.36103/ijas.v54i1.1702
33.Yousefnia, S., D. Naseri, F. Seyed Forootan, M. Tabatabaeia, F. Moattar, T. Ghafghazi and *et al*. 2020. Suppressive role of

Viola odorata extract on malignant characters of mammosphere-derived breast cancer stem cells. Clinical and Translational Oncology, 22:1619-1634.
DOI: 10.1007/s12094-020-02307-9.

34.Yuan, M., Y. Zhao, H. T. Arkenau, T. Lao, L. Chu and Q. Xu. 2022. Signal pathways and precision therapy of small-cell lung cancer. Signal Transduction and Targeted Therapy, 7(1):187.
DOI: 10.1038/s41392-022-01013-y3

35.Zagaja, M., A. Zagaja, J. Szala-Rycaj, A. Szewczyk, M. K. Lemieszek, G. Raszewski and *et al*. 2022. Influence of umbelliferone on the anticonvulsant and neuroprotective activity of selected antiepileptic drugs: An in vivo and in vitro study. International Journal of Molecular Sciences, 23(7):3492.
DOI: 10.3390/ijms23073492

36.Zhang, L., Q. Xie and X. Li. 2022. Esculetin: A review of its pharmacology and pharmacokinetics. Phytotherapy Research, 36(1):279-98. DOI: 10.1002/ptr.7311