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Iris spuria hydroalcoholic extract decreases the proliferation and migration of U87 glioblastoma and HepG2 liver cancer cells

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Abstract

Objective: Studies suggested that herbal compounds from the Iridaceae family have anticancer potential. The present study aimed to test the impact of *Iris spuria* on U87 glioblastoma and HepG2 liver cancer cells.

Materials and methods: The cells were treated with hydroalcoholic extract of *I. spuria* rhizome for 24 or 48 hr. The MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) test and propidium iodide staining were performed to evaluate cell proliferation and cell cycle analysis, respectively. The cell migration was examined by scratch assay. Oxidative stress was determined by assessing the levels of reactive oxygen species (ROS) and malondialdehyde (MDA).

Results: The extract showed antiproliferative effect in time- and concentration-dependent manner. The liver cancer cells, glioblastoma cells and fibroblasts (L929 cells as control) were respectively more sensitive to this effect. The extract at concentrations of ≥ 100 µg/ml could rise the levels of ROS and MDA and increase the percentage of cells in the sub-G1 stage of the cell cycle. In addition, the extract at 6-25 µg/ml significantly decreased the migration rate of HepG2 cells. Liquid chromatography-mass spectrometry revealed the presence of at least 19 compounds such as flavonoids and sesquiterpenoids in the extract.

Conclusion: Hydroalcoholic extract of *I. spuria* showed cytotoxic activity against liver cancer and glioblastoma cells by inducing oxidative stress. Future studies should focus on isolating the active ingredients of the extract to induce anticancer effects at lower concentrations.

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Introduction

Nowadays, conventional therapeutic approaches against cancer include surgery, radiotherapy, and adjuvant chemotherapy. Also, other treatment methods are being developed, which include stem cell therapy, targeted therapy, nanoparticles, radionics, and chemodynamic therapy (Debela et al. 2021).

Compounds derived from medicinal plants are always good candidates for developing new medicines. Numerous experimental and clinical works have demonstrated the anticancer properties of various plant-based compounds (Buyel 2018; Hosseini and Ghorbani 2015; Shaik et al. 2022). In fact, several drugs currently utilized in clinical setting have been discovered from plant sources, examples of which are: taxol, camptothecin, vinca alkaloids, and podophyllotoxins (Moraes et al. 2017). Therefore, extensive research on plant compounds is warranted to find new anticancer drugs.

Iris spuria L. is a rhizomatous perennial plant in the family Iridaceae that grows in different regions of the world. Experimental studies have revealed that various *Iris* species exhibit beneficial effects against a range of diseases including inflammation and bacterial and viral infections (Alam 2017; Liu et al. 2022; Michalak et al. 2021; Tuyiringire et al. 2020). Previous studies also suggested that plants from the Iridaceae family have the potential for anticancer effects. For example, *Iris hungarica, Crocus sativus, Moraea sisyrinchium,* and *Iris taochia* showed cytotoxic activity against different cancer cell lines (King et al. 2023; Mykhailenko et al. 2020; Rashidi et al. 2024; Yazgan et al. 2022). Notably, methanolic extract of *Iris barnumiae* induced anti-proliferative activity on HT29 colon cancer, MCF-7 breast cancer, PC3 prostate carcinoma, and U87MG glioma cells (Abdullah 2024). Regarding *I. spuria*, its pharmacological effects have not been well studied so far. It was reported that rhizomes of this plant protects the liver against paracetamol-induced toxicity (Akther et al. 2014). Also, tectorigenin glycosides isolated from its rhizome could show nephroprotective activity *in vitro* (Divya et al. 2021). Given the lack of research on the antitumor potential of *I. spuria*, the current study aims to test the effects of its hydroalcoholic extract on cancerous cells proliferation and migration.

Materials and Methods

Materials

The glioblastoma multiform cells (U87), liver cancer cells (HepG2), and mouse fibroblast (L929) were provided from Pasteur Institute (Iran). MTT (purity ≥ 98%), sodium citrate (purity ≥ 98%), triton X-100, propidium-iodide (purity ≥ 94%), and thiobarbituric acid (purity ≥ 98%) were purchased from Sigma-Aldrich (USA). Penicillin-streptomycin, fetal bovine serum (FBS), and high glucose Dulbecco's Modified Eagle's medium (DMEM) were purchased from Gibco (USA). Trypsin-EDTA was obtained from BIO-IDEA, Iran. 2',7'-dichlorofluorescin diacetate (DCFH-DA, purity ≥ 97%) and dimethyl sulfoxide (purity ≥ 99.9%) were purchased from Pars Tous Biotech (Iran).

Preparation of hydroalcoholic extract of *I. spuria*

Iris spuria L. was collected from the farm of the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. A plant sample was kept at the herbarium of this university (voucher specimen number: 40515FUMH). After drying the plant rhizome in the shade, it was grounded into a fine powder. The maceration extract was obtained by pouring 90 g of the powder in 400 ml of ethanol (70% v/v). The suspension was kept in an oven at 37°C for 3 days and was shaken each 12 hr. In order to eliminate insoluble particles, the extract was centrifuged at 1500 rpm for 5 min. Then, the supernatant was filtered using filter paper and a vacuum pump and it was dried at 37°C. The dried hydroalcoholic

extract was kept frozen until use and the yield was 13%.

Cell culture

The U87, HepG2, and L929 cells were maintained in DMEM containing 1% penicillin-streptomycin and 10% FBS. The 96-well culture plates were used for evaluation of cell proliferation and reactive oxygen species (ROS). The cells were treated with the plant extract (25-400 µg/ml) for 24 or 48 hr. Doxorubicin was used as a reference drug. Dimethyl sulfoxide with a concentration of 0.5% was added to the culture medium of untreated cells as vehicle control. For analysis of apoptosis and lipid peroxidation (malonaldehyde (MDA) assay), the 12-well plates were used and the cells were treated for 24 hr with the extract.

Cell proliferation

The effect of the plant extract on proliferation was evaluated with MTT test, a colorimetric test for measuring the conversion of MTT to formazan crystals by dehydrogenases occurring in the mitochondria of living cells. After treatment with the extract, the medium of culture plate was replaced by a fresh DMEM (90 µL per well) and 10 µL of MTT reagent (10 mg/ml) was added to each well. After 4 hr, the medium was removed and the formazan attached to the bottom of each well was dissolved in 200 µl dimethyl sulfoxide. Then, the absorbance of the formazan solution was evaluated at 570 and 630 nm (background) by a Stat FAX – 2100 plate reader (Awareness Technology Inc., USA).

Propidium iodide staining

The effect of hydroalcoholic extract of *I. spuria* on the cell cycle was measured with propidium iodide, a fluorescent dye that intercalates into DNA and shows the amount of fragmented DNA. The apoptotic cells contain more fragmented DNA and display proportionally raised fluorescence intensity. Following incubation with the extract (24 hr), the U87 and HepG2 cells were collected by trypsin. Then, a solution containing propidium iodide (50 µg/ml) was added to the cells. This solution also contained sodium citrate (0.1%), RNAse (50-100 µg/ml), and Triton-X 100 (0.1%) to make cell membrane permeable. After 30 min, the fluorescence was measured with a flow cytometer (FACSCALIBUR™, Becton Dickinson, CA, USA). The FlowJo ® vX.0.7 software was used to analysis data (Tree Star, Ashland, OR, USA).

Measuring the level of ROS

The DCFH-DA reagent was used to determine the level of ROS in HepG2 and U87 cells. In the presence of ROS, DCFH is changed to dichlorofluorescein (a highly fluorescent compound). The cells were incubated for 24 hr with the plant extract. After washing with PBS, the cells were incubated with 20 µM of DCFH-DA for 30 min at 37°C. Then, the fluorescence severity was evaluated with the flow cytometer at an excitation/emission of 485/530 nm.

Evaluation of lipid peroxidation

The level of MDA, the final compound of the peroxidation reaction, was determined in HepG2 and U87 cells. The treated (24 hr) cells were collected using trypsin, homogenized in 1.15% KCl solution, and centrifuged for 10 min at 15000 rpm (4°C). Next, 800 µl of thiobarbituric acid (0.7%) and 500 µl of trichloroacetic acid (15%) were added to 500 µl of supernatant. After boiling the mixture for 60 min, it was centrifuged at 1500 rpm for 10 min. The fluorescence was evaluated at an excitation/emission wavelengths of 530/550 nm.

Scratch assay

The scratch method was used to evaluate the effect of plant extract on migration capability of HepG2 and U87 cells. The U87 cells were cultured in a 12-well plate to reach a confluent monolayer. Using a sterile pipette tip, a linear scratch

was applied in the cell culture. Then, the ruptured cells were washed with PBS. The cells were treated for 48 hr with 6-25 μg/ml of the plant extract. The assay was carried out in triplicate and microscopic photographs were analyzed by Image J software to measure the distance between the two sides of the scratched area.

Liquid chromatography-mass spectrometry (LC-MS)

The LC-MS test was done using an Agilent 1200 series liquid chromatography connected to an Agilent 6410 triple quadrupole mass spectrometer. The separation of the plant extract compounds was performed on a C18 column (Agilent Eclipse Plus) using a mobile phase containing water + 0.1% formic acid (A) methanol + 0.1% formic acid (B). The gradient program was as follows: 0 - 1 min 10% B, 1 - 40 min from 10% to 100% (B), 40 - 42 min 100% (B), and 42 - 50 min from 100% to 10% (B). The mass spectra were obtained in the range of 100 to 1000 with negative electrospray ionization mode and the following MS parameters: capillary voltage = 4.0 kV; nebulizer pressure = 35 psi; drying gas (N_2) flow rate = 10 L/min; drying gas temperature = 350°C and 50 min scan time. The MZmine software was used for peak extraction and analysis of the data. Compound identification was performed by comparing the measured accurate mass, MS fragmentation patterns, and chromatographic retention behavior with those reported in literature and database sources.

Statistical analysis

To compare the difference between control and treatment cells, one-way analysis of variance (ANOVA) and Tukey's post hoc test were used. A $p<0.05$ level was considered significant and data are shown as mean ± SEM.

Results

Effects of *I. spuria* on the cell proliferation

As presented in Figure 1A, *I. spuria* extract had no cytotoxic activity on nonmalignant L929 cells at levels up to 100 μg/ml after 24 hr (IC_{50} = 320 μg/ml) and 48 hr (IC_{50} = 160 μg/ml).

The extract significantly reduced the viability of U87 cells after 24 and 48 hr at concentrations of ≥ 200 μg/ml ($p<0.01$) and ≥ 25 μg/ml ($p<0.001$), respectively (Figure 1B). The IC_{50} value of the extract was 300 μg/ml and 130 μg/ml after 24 and 48 hr, respectively. The toxic concentration of the extract was much higher than that of doxorubicin (0.37-3 μg/ml, $p<0.001$) as a reference drug.

In the case of HepG2 cells, *I. spuria* extract showed an antiproliferative effect at concentrations of ≥ 100 μg/ml after 24 and 48 hr (Figure 1C, $p<0.001$). This effect showed a concentration-dependent behavior and the IC_{50} value of the extract was 170 and 106 μg/ml after 24 and 48 hr, respectively. Doxorubicin, decreased the HepG2 cell viability at concentrations of ≥ 0.37 μg/ml ($p<0.001$) after 48 hr. The extract at concentrations of 100 to 400 μg/ml could increase the antiproliferative activity of doxorubicin (Figure 2).

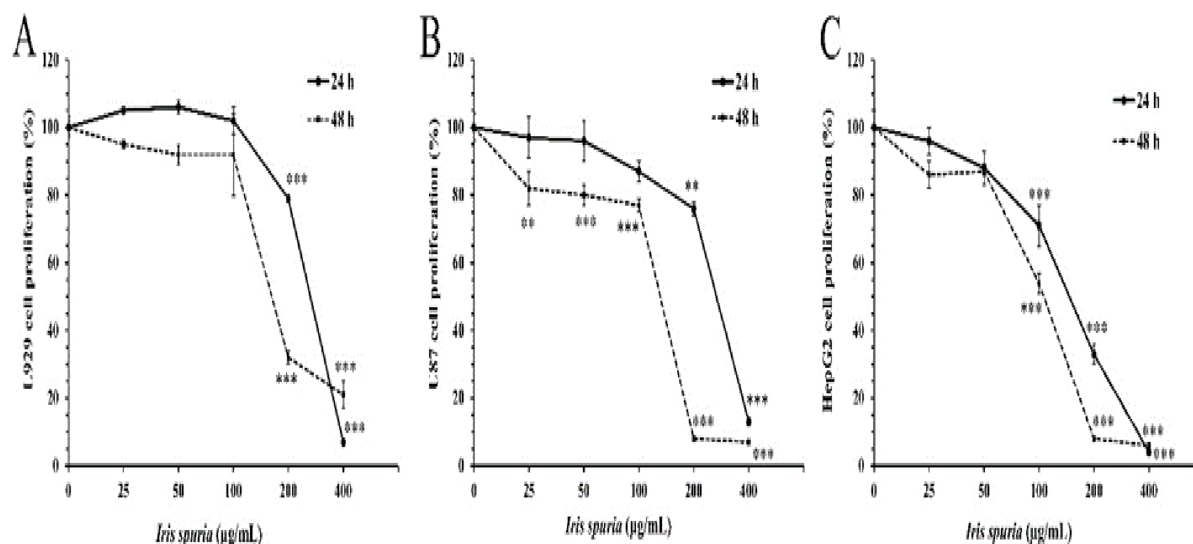

Figure 1. Effects of hydroalcoholic extract of *Iris spuria* on the proliferation of L929 mouse fibroblast (A), U87 glioblastoma cells (B), and HepG2 liver cancer cells (C). The cells were incubated for 24 or 48 hr with the plant extract. Data are presented as mean ± SEM (n = 4). **p<0.01 and ***p<0.001 compared to untreated cells (concentration of 0 μg/ml).

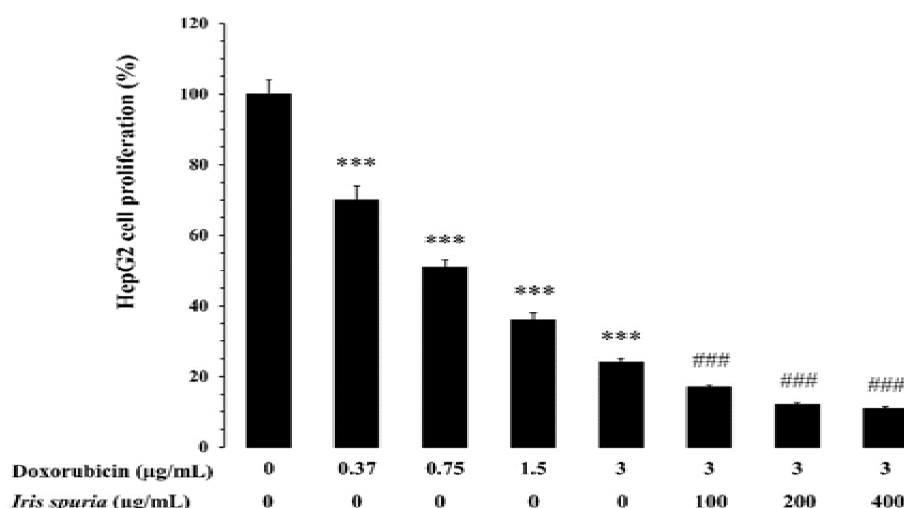

Figure 2. Effects of co-incubation with hydroalcoholic extract of *Iris spuria* and doxorubicin on the proliferation of HepG2 cells. The cells were incubated for 48 hr with the plant extract and/or doxorubicin. Data are presented as mean ± SEM (n = 4). ***p<0.001 compared to untreated cells (concentration of 0 µg/ml) and ###p<0.001 compared to only doxorubicin-treated cells.

Effect of *I. spuria* on cell cycle

Treatment of U87 cells (24 hr) with the extract enhanced the proportion of cells in the sub-G1 phase. This effect was observed in concentrations of 200 and 400 µg/ml (p<0.001) of the extract (Figure 3). In the case of HepG2 cells, the impact of the extract on the sub-G1 stage was tested at concentrations of 25-400 µg/ml and it was significant at ≥ 100 µg/ml (Figure 4).

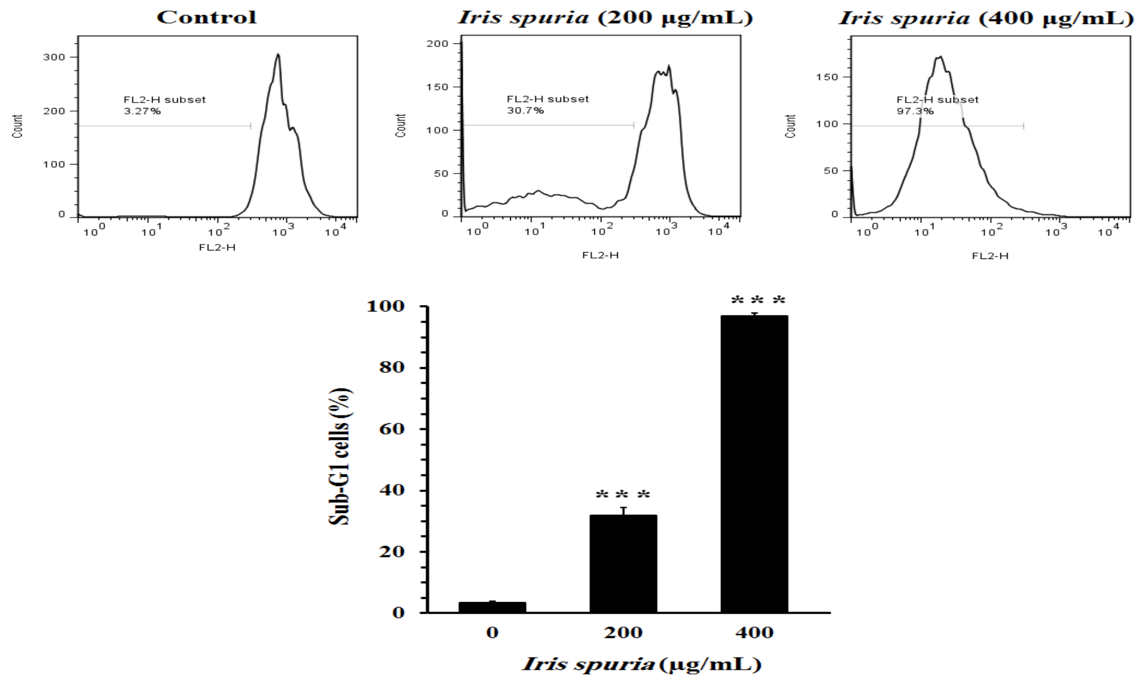

Figure 3. Effect of hydroalcoholic extract of *Iris spuria* on the cell cycle of U87 cells. A: Representative histogram of propidium iodide-stained cells treated for 24 hr with hydroalcoholic extract of *Iris spuria.* B: Quantitative analysis of cell population in the sub-G1 stage following treatment with the extract. Data are presented as mean ± SEM (n = 3). ***p<0.001 compared to untreated cells (concentration of 0 µg/ml).

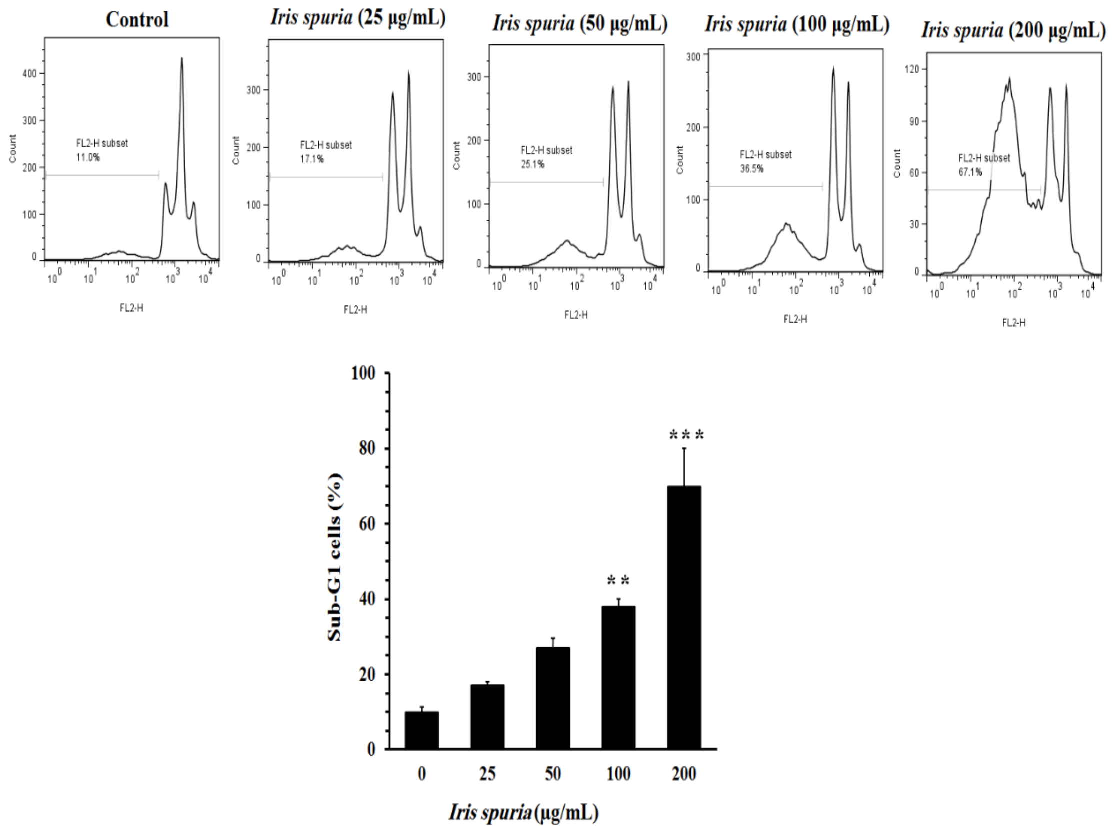

Figure 4. Effect of hydroalcoholic extract of *Iris spuria* on the cell cycle of HepG2 cells. A: Representative histogram of propidium iodide-stained cells treated for 24 hr with hydroalcoholic extract of *Iris spuria.* B: Quantitative analysis of cell population in the sub-G1 stage following treatment with the extract. Data are presented as mean ± SEM (n = 3). **p<0.01 and ***p<0.001 compared to untreated cells (concentration of 0 µg/ml).

Effect of *I. spuria* on ROS level

Treatment of U87 cells for 24 hr with *I. spuria* extract significantly ($p<0.01$) enhanced the content of intracellular ROS at 200 and 400 µg/ml. The extract in low concentration (25 µg/ml) did not increase the ROS level, but decreased it (Figure 5A).

The effect of the extract on HepG2 cells was started at a concentration of 50 µg/ml (Figure 5B). Incubation for 24 hr increased the ROS level at concentrations of 100, 200, and 400 µg/ml to 2.7, 3.9, and 5.4 times that of the control, respectively.

Effects of *I. spuria* on MDA level

As shown in Figure 6, in both U87 and HepG2 cells, the plant extract significantly increased the amount of MDA after 24 hr. This effect was concentration-dependent and the percent of MDA was higher in HepG2 cells than in U87 cells.

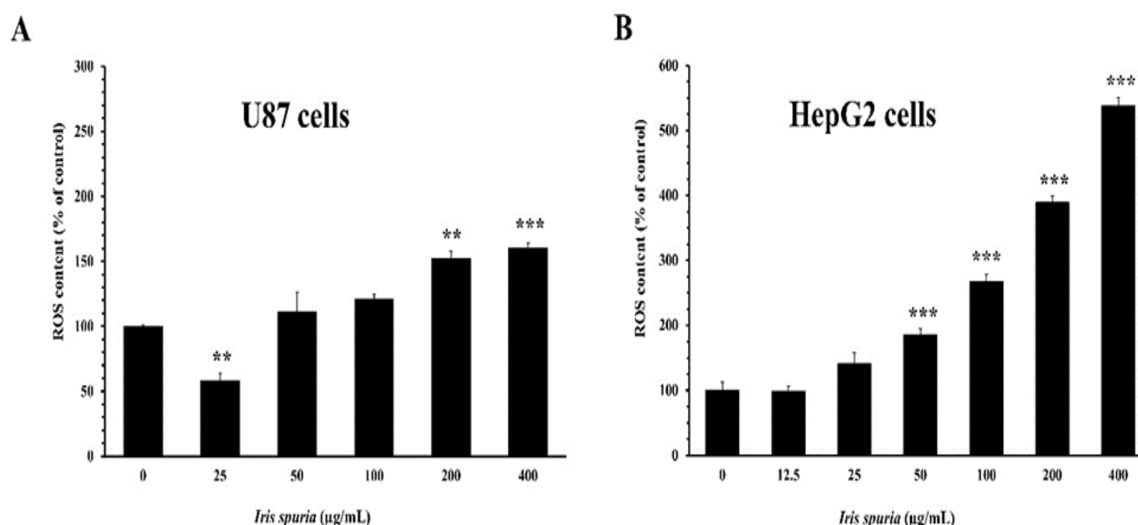

Figure 5. Effects of hydroalcoholic extract of *Iris spuria* on the level of reactive oxygen species (ROS) in U87 cells (A) and HepG2 (B) cells. The cells were incubated for 24 hr with the plant extract. Data are presented as mean ± SEM (n = 4). **p<0.01 and ***p<0.001 compared to untreated cells (concentration of 0 µg/ml).

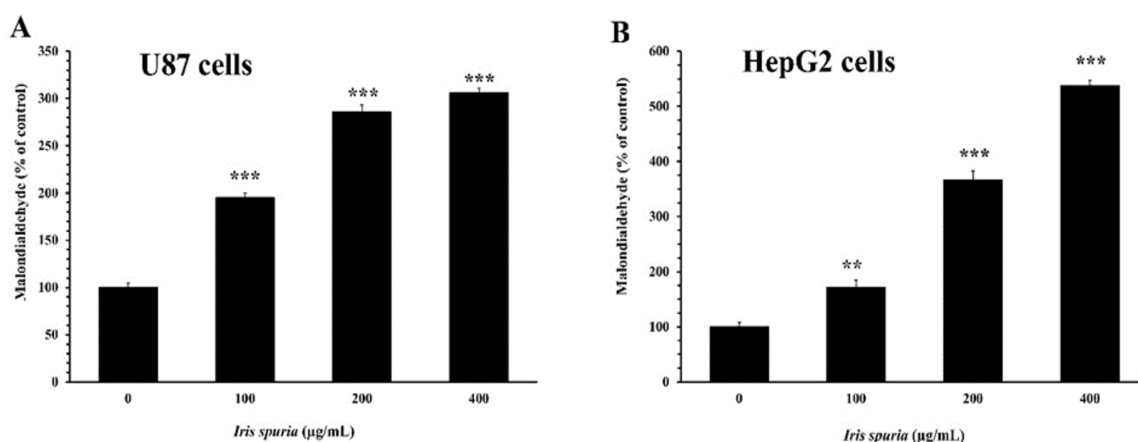

Figure 6. Effects of hydroalcoholic extract of *Iris spuria* on the content of malondialdehyde (MDA) in U87 cells (A) and HepG2 (B) cells. The cells were incubated for 24 hr with the plant extract. Data are presented as mean ± SEM (n = 3). **p<0.01 and ***p<0.001 compared to untreated cells (concentration of 0 µg/ml).

Effects of *Iris spuria* on cell migration

Figure 7 shows the effects of non-cytotoxic concentrations of the extract on the migration of HepG2 cells. In untreated (control) cells, the scratch width between cells was significantly decreased after 24 hr and completely disappeared after 48 hr ($p<0.01$). The extract (6, 12.5, and 25 µg/ml) remarkably reduced the cell migration ability and the scratched gap remained open even after 48 hr.

LC-MS analysis of hydroalcoholic extract of *I. spuria*

Figure 8 shows the total ion chromatogram of the extract of *I. spuria* rhizome. Data of MS spectral were compared with known phytochemicals from previous studies. The comparison revealed the presence of at least 19 compounds in the extract (Table 1). These compounds were from different phytochemical classes such as flavonoids (luteolin, iristectorigenin, isorhamnetin, tectorigenin, and apigenin), sesquiterpenoids (amorphene), and fatty acids (palmitic acid and stearic acid).

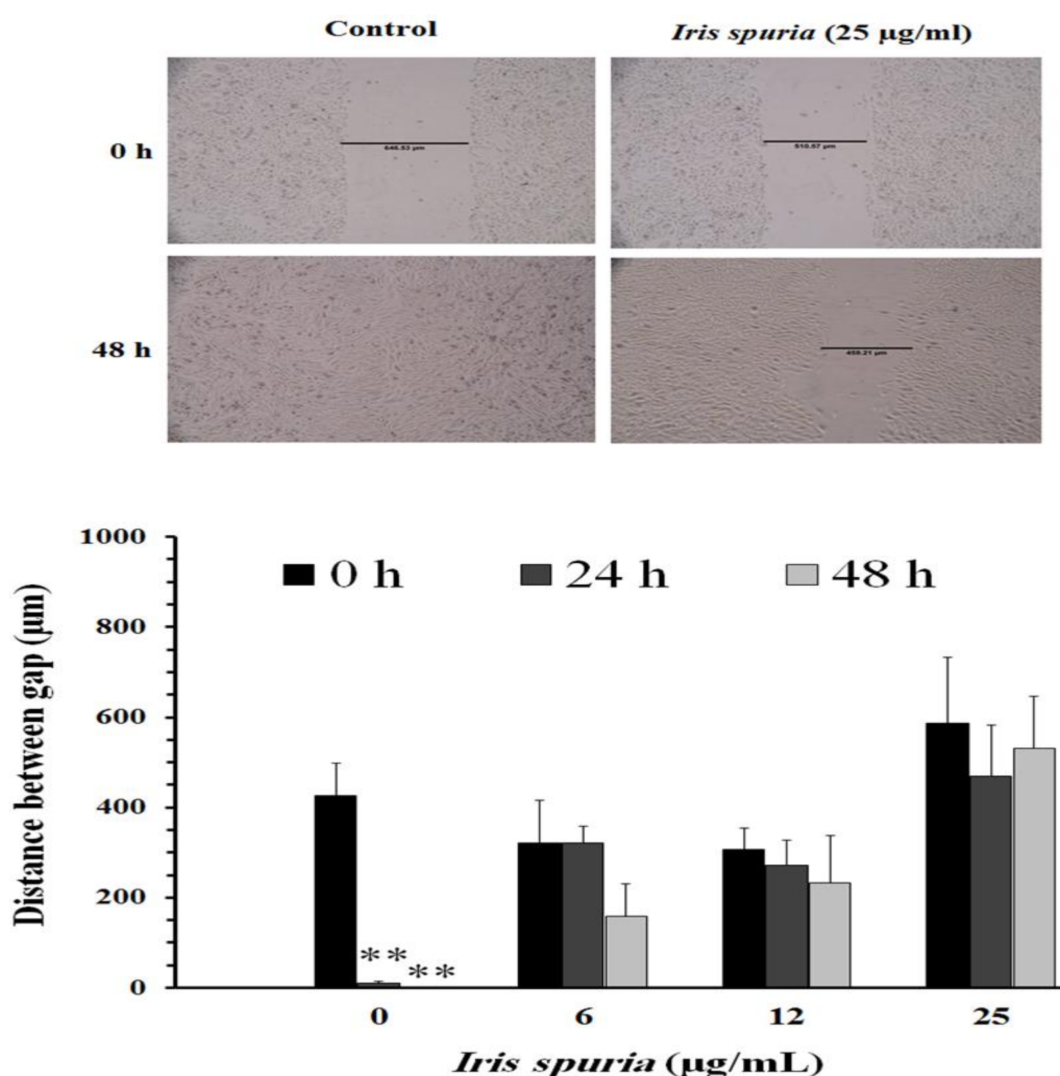

Figure 7. Effects of hydroalcoholic extract of *Iris spuria* on the migration of HepG2 cells. A: Representing photomicrographs of the cells before and after (48 hr) treatment with the plant extract. B: Quantitative analysis of the distance between the two sides of the scratched area. Data are presented as mean ± SEM (n = 3). $^{**}p<0.01$ compared to time 0 (before treatment).

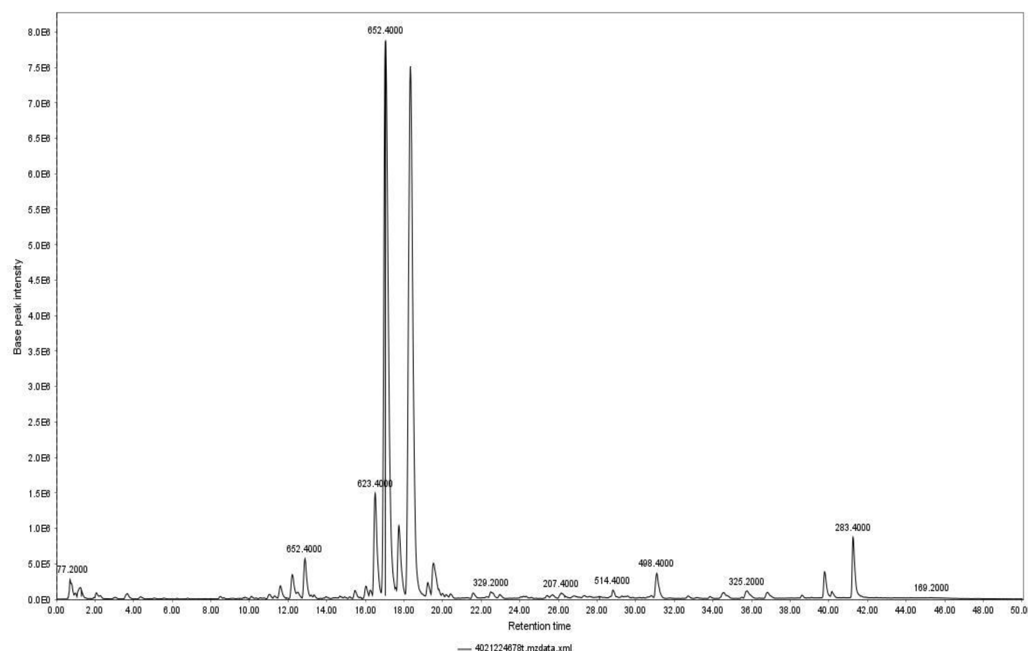

Figure 8. Total ion chromatogram of hydroalcoholic extract of *Iris spuria* rhizome

Table 1. Phytochemical composition of hydroalcoholic extract of *Iris spuria* rhizome. The LC-MS was performed in negative electrospray ionization mode.

Peak No.	Compound identification	Molecular formula	t_R (min)	[M-1] (*m/z*)	Reference
1	A/5,40-Methoxy-6,7-methylenedioxy-isoflavone-30 -O-βd-glucopyranoside	$C_{24}H_{24}O_{12}$	0.73	503	Hoang et al. 2020
2	C/Iriskashmirianin 4’,5-dimethoxy-3-hydroxy-6,7-methylenedioxyisoflavone Nigricanin	$C_{18}H_{14}O_7$	1.156	341	
3	α-Dehydroirigermanal	$C_{30}H_{48}O_3$	2.186	455	
4	4-O-methyliriflophenone	$C_{14}H_{12}O_5$	3.099	259	Hoang et al. 2020;
5	3-O-glucoside /Luteolin 6-C-glucoside/ Luteolin 8-C-hexoside	$C_{21}H_{20}O_{11}$	3.099	447	Kostić et al. 2019
6	Irisdichotin B	$C_{23}H_{26}O_{12}$	3.099	493	Kostić et al. 2019
7	Vanillic acid	$C_8H_8O_4$	3.641	167	Hoang et al. 2020
8	Palmitic acid	$C_{16}H_{32}O_2$	3.641	255	
9	Iristectorigenin B/Iristectorigenin A	$C_{17}H_{14}O_7$	3.641, 39.814	329	Hoang et al. 2020; Kostić et al. 2019
10	7-O-Gluco-luteolin	$C_{21}H_{20}O_{12}$	3.641	464	Sulaiman and Balachandran, 2016
11	Amorphene/α-Muurolene/β-Gurjuenene/γ-Elemene	$C_{15}H_{24}$	4.357	203	Hoang et al. 2020
12	Isorhamnetin 3-O-(2“-hamnosyl) hexoside/ Isorhamnetin 3-O-(6“-rhamnosyl)	$C_{28}H_{32}O_{16}$	12.277, 14.771, 16.533	623	Hoang et al. 2020; Kostić et al. 2019
13	Tectorigenin-40 -O-diglucoside/ Tectorigenin-7-O-diglucoside	$C_{28}H_{32}O_{17}$	16.02	639	Hoang et al. 2020
14	Swertiajaponin/Tectoridin	$C_{22}H_{22}O_{11}$	17.752	461	Hoang et al. 2020;
15	Iristectorigenin A 7-O-hexuronide/ Irisdichotin A/ Iristectoridin B/ Iristectorin A/ Iristectorin B	$C_{23}H_{24}O_{12}$	17.752, 18.357	491	Kostić et al. 2019
16	Iristectorigenin B/ Iristectorigenin A	$C_{17}H_{14}O_7$	28.826	329	
17	Apigenin-6,8-di-C-arabinoside	$C_{25}H_{26}O_{13}$	31.130	533	Hoang et al. 2020
18	Nigracin 4'-O-[6"-(3-hydroxy-3-methylglutaryl)]-hexoside	$C_{30}H_{33}O_{16}$	31.130	647	Kostić et al. 2019
19	Stearic acid	$C_{18}H_{36}O_2$	41.269	283	Hoang et al. 2020

Discussion

This study showed that hydroalcoholic extract of rhizome of *I. spuria* reduces the proliferation and survival of malignant cells. The antiproliferative effect was in time- and concentration-dependent manner. Analysis of IC_{50} values revealed that liver cancer cells, glioblastoma cells, and fibroblasts were respectively more sensitive to this effect, recommending that malignant cells are more sensitive to the antiproliferative activity of *I. spuria*. Specifically, the HepG2 cells showed more suppression in their proliferation compared to U87 cells. However, even for HepG2 cells, the IC_{50} value (106 µg/ml, 48 hr) of *I. spuria* was higher than the level suggested by the American National Cancer Institute for anticancer plant extracts (concentration < 30 µg/ml) (Canga et al. 2022). In addition, compared to doxorubicin, the antiproliferative effect of *I. spuria* on HepG2 occurred at higher concentrations. Therefore, the antiproliferative effect of *I. spuria* is valuable clinically when it works in lower concentrations. Isolation of active extract compounds could potentially make more potent agents available that work at lower doses.

Doxorubicin is a type of chemotherapy agent that traps topoisomerase enzymes (induces DNA damage), disrupts mitochondria function, and increases the formation of intracellular ROS. Therefore, depending on the dosage, doxorubicin can trigger senescence, apoptosis, autophagy, pyroptosis, or necrosis in cancer cells (Kciuk et al. 2023). Despite these beneficial effects, the cardiotoxicity associated with doxorubicin limits its broader application in cancer treatment. Strategies to mitigate this risk include reducing the required dose of doxorubicin or using compounds that enhance the drug efficacy which is somewhat helpful in decreasing the risk of cardiotoxicity (Mody et al. 2023). Our data indicate that hydroalcoholic extract of *I. spuria* enhanced the antiproliferative activity of doxorubicin and this encourages future studies on the active ingredients of this plant. Supporting this hypothesis, the extract increased the level of ROS and lipid peroxidation, and induced cell cycle arrest. The cellular arrest was visible as a rise in the number of cells in the sub-G1 phase that indicates the occurrence of apoptosis or mitosis arrest due to oxidative stress.

One of the significant challenge in cancer therapy is relocation of malignant cells to distant and nearby tissues (Fares et al. 2020). In the present work, the activity of *I. spuria* was tested on the migration ability of HepG2 cells. *Iris spuria* extract demonstrated the ability to inhibit cell migration at non-cytotoxic concentrations. Cancer cell invasion involves two main patterns: collective cell migration or individual cell migration. During migration process, tumor cells overcome the extracellular matrix barriers and penetrate the surrounding tissues with different biochemical/molecular mechanisms (Krakhmal et al. 2015). The inhibitory effect of *I. spuria* extract on cell migration may be due to its action on cytoskeleton, adhesion molecules, or matrix metalloproteins (Merino-Casallo et al. 2022). The exact mechanisms of this effect should be clarified in future studies.

Experimental studies have shown that several kind of plant-isolated ingredients including polyphenols, flavonoids, alkaloids, and terpenes prevent initiation and invasion of cancer (El Omari et al. 2021; Sheikh et al. 2020). Our LC-MS analysis suggested the presence of various phytochemicals including flavonoids and sesquiterpenoids in *I. spuria* extract. In line with our findings, previous studies have shown that *I. spuria* has multiple flavonoids such as tectorigenin, apocynin, irigenin, and iridin (Bhat et al. 2014; Divya et al. 2021; Khallaf et al. 2021). Although it is difficult to determine which of the ingredient is responsible for the antitumor activity of this plant, other studies reported the anticancer effects of tectorigenin, apocynin, irigenin, and iridine on various cancer cells including liver cancer and glioblastoma (Bhosale et al. 2021; Jantaree

et al. 2017; Xu et al. 2022; Yeh et al. 2020). Additionally, by reviewing the published articles, we have compiled a list of compounds from *I. spuria* that can be responsible for the cytotoxic effect against cancer cells (Table 2). In support of our findings, previous studies have reported the anticancer effect for other species of *Iris*. For example, it has been reported that flavonoids of *Iris tectorum* induced cytotoxicity in MCF-7 breast cancer cells and C32 melanoma cells (Fang et al. 2008). Another study reported antiproliferative activity for methanolic extracts of *Iris spuria, Iris kashmiriana, Iris germanica, and Iris ensata* against A549 lung cancer and Caco-2 colon cells (Wani et al. 2017).

One of the limitation of our work was that the exact mechanisms responsible for the cytotoxicity of *I. spuria* was not determined. Previous studies have determined several intracellular pathways that regulate cell cycle progression, proliferation, autophagy, apoptosis, angiogenesis, and invasion of cancer cells. Among these pathways are the PI3K-Akt-mTOR, NF-κB, and MAPK (mitogen-activated protein kinase) cascades (Dai et al. 2015; Jantaree et al. 2017; Trejo-Solís et al. 2018; Zeng et al. 2018). Therefore, further studies can focus on the effect of *I. spuria* on these signaling pathways. Another limitation of our study are the lack of normal cell for comparison of migration assay and the absence of apoptosis confirmation (e.g. annexin V staining).

In conclusion, hydroalcoholic extract of *I. spuria* rhizome inhibited the proliferation and cell cycle of hepatocellular carcinoma and glioblastoma cells by inducing oxidative stress. The liver cancer cells showed more suppression in their proliferation compared to U87 cells. The *I. spuria* rhizome extract by the way conducted in this study shows potential as a complementary compound in cancer management, particularly when its active components can be harnessed effectively. Future studies should focus on isolating the active ingredients of the extract to show anticancer effects at lower concentrations. Also, more studies should be done on other cancer cells and animal studies. Multiple mechanisms involved in the cytotoxicity pathway need to be identified.

Table 2. Anticancer effects reported for phytochemicals present in *Iris spuria*.

Phytochemical	Anticancer effect	Cell line	Reference
Tectorigenin	Breast cancer	MDA-MB-231 and MCF-7	Zeng et al. 2018
	Liver cancer	HepG2	Jiang et al. 2012
	Glioblastoma	GBM-8401 and GBM-8901	Yeh et al. 2020
	Prostate cancer	LNCaP	Thelen et al. 2006
	Colon cancer	Caco-2	Dai et al. 2015
Vanillic acid	Colon cancer	HCT116	Kaur et al. 2021
Palmitic acid	Gastric cancer	MGC-803	Wang et al. 2025
	Liver cancer	HepG2	
	Prostate cancer	PC3	
	Breast cancer	BT474	
	Colon cancer	HCT116	
Elemene	Breast cancer	MCF-7	Tan et al. 2021
	Lung cancer	PC-9 cells	
	Gastric cancer	SGC-7901	
Swertiajaponin	Melanoma	B16F10	Lee et al. 2017
Tectoridin	Colon cancer	HCT116	Xiong et al. 2021
	Ovarian cancer	*SK-OV-3*	Wang and Shi 2020
	Breast cancer	MCF-7	Kang et al. 2009
Stearic acid	Breast cancer	Hs578t	Evans et al. 2009

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Conflicts of interest

The authors had no competing interests.

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Ethical Considerations

This study was approved by the Research Ethics Committee of Mashhad University of Medical Sciences.).

Code of Ethics

IR.MUMS.MEDICAL.REC.1400.325

Authors' Contributions

AG proposed the idea of study and supervised the experiments. RR conducted culture of cells and their treatments and wrote the initial draft of paper. FS contributed to experimental works. EE conducted LC-MS analysis. It is hereby acknowledged that all authors have accepted responsibility for the manuscript's content and consented to its submission.

Abbreviations

2',7'-dichlorofluorescin diacetate: DCFH-DA, 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide: MTT, Dulbecco's Modified Eagle's medium: DMEM, Fetal bovine serum: FBS, Malondialdehyde: MDA, One-way analysis of variance: ANOVA, Reactive oxygen species: ROS

References

Abdullah FO (2024) Phytochemical identification by LC-ESI MS/MS method of the Iris barnumiae methanolic extract and its antiproliferative and apoptosis-inducing effects. Biomass Convers. Biorefinery 14(24):30983-30995

Akther N, Andrabi K, Nissar A ,et al. (2014) Hepatoprotective activity of LC-ESI-MS standardized Iris spuria rhizome extract on its main bioactive constituents. Phytomedicine 21(10):1202-7

Alam A (2017) Pharmacology and phytochemistry of isoflavonoids from Iris species. J Pharmacol Clin Res 3:1-6

Bhat G, Shawl AS, Shah Z, Tantry M (2014) HPLC-DAD-ESI-MS/MS identification and characterization of major constituents of Iris crocea, Iris germanica and Iris spuria growing in Kashmir Himalayas, India. J Anal Bioanal Tech 5(6):1-10

Bhosale PB, Vetrivel P, Ha SE, et al. (2021) Iridin induces G2/M phase cell cycle arrest and extrinsic apoptotic cell death through PI3K/AKT signaling pathway in AGS gastric cancer cells. Molecules 26(9)

Buyel JF (2018) Plants as sources of natural and recombinant anti-cancer agents. Biotechnol Adv 36(2):506-520

Canga I, Vita P, Oliveira AI, Castro MA, Pinho C (2022) In vitro cytotoxic activity of african plants: a review. Molecules 27(15):4989.

Dai X-Y, Yu Y-M, Kong Z-F, et al. (2015) Tectorigenin inhibits Caco-2 human colon cells via NF-κB pathway suppression. Bangladesh J Pharmacol 10(4):948-955

Debela DT, Muzazu SG, Heraro KD, et al. (2021) New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med 9:20503121211034366

Divya G, Albert A, Singab ANB, et al. (2021) Renoprotective effect of tectorigenin glycosides isolated from Iris spuria L. (Zeal) against hyperoxaluria and hyperglycemia in NRK-49Fcells. Nat Prod Res 35(6):1029-1034

El Omari N, Bakrim S, Bakha M, et al. (2021) Natural bioactive compounds targeting epigenetic pathways in cancer: a review on alkaloids, terpenoids, quinones, and isothiocyanates. Nutrients 13(11) 3714

Evans LM, Cowey SL, Siegal GP, Hardy RW (2009) Stearate preferentially induces apoptosis in human breast cancer cells. Nutr Cancer 61(5):746-53

Fang R, Houghton PJ, Hylands PJ (2008) Cytotoxic effects of compounds from Iris tectorum on human cancer cell lines. J Ethnopharmacol 118(2):257-63

Fares J, Fares MY, Khachfe HH, Salhab HA,

Fares Y (2020) Molecular principles of metastasis: a hallmark of cancer revisited. Signal Transduct Target Ther 5(1):28

Hoang L, Beneš F, Fenclová M, et al. (2020) Phytochemical composition and in vitro biological activity of Iris spp.(Iridaceae): A new source of bioactive constituents for the inhibition of oral bacterial biofilms. Antibiotics *9*(7):403

Hosseini A, Ghorbani A (2015) Cancer therapy with phytochemicals: evidence from clinical studies. Avicenna J Phytomed 5(2):84-97

Jantaree P, Lirdprapamongkol K, Kaewsri W, et al. (2017) Homodimers of vanillin and apocynin decrease the metastatic potential of human cancer cells by inhibiting the FAK/PI3K/Akt signaling pathway. J Agric Food Chem 65(11):2299-2306

Jiang CP, Ding H, Shi DH, Wang YR, Li EG, Wu JH (2012) Pro-apoptotic effects of tectorigenin on human hepatocellular carcinoma HepG2 cells. World J Gastroenterol 18(15):1753-64

Kang K, Lee SB, Jung SH, et al. (2009) Tectoridin, a poor ligand of estrogen receptor alpha, exerts its estrogenic effects via an ERK-dependent pathway. Mol Cells 27(3):351-7

Kaur J, Gulati M, Gowthamarajan K, et al. (2021) Combination therapy of vanillic acid and oxaliplatin co-loaded in polysaccharide based functionalized polymeric micelles could offer effective treatment for colon cancer: A hypothesis. Med Hypotheses 156:110679

Kciuk M, Gielecińska A, Mujwar S, et al. (2023) Doxorubicin-An agent with multiple mechanisms of anticancer activity. Cells 12(4)

Khallaf ISA, Bayoumi SAL, Malak LG, et al. (2021) The inhibition of inducible nitric oxide production in lipopolysaccharide-stimulated rat macrophages and in silico studies by flavonoids from Iris spuria L. rhizomes. J Ethnopharmacol 278:114311

King T, Moscardini S, Martin M, Rehman FK (2023) Saffron (Crocus sativus) extract inhibits migration and invasion of cancer cells through modulation of MMP-3 and MMP-9 expression. Cancer Research 83(7_Supplement):1319-1319

Kostić AŽ, Gašić UM, Pešić MB, et al. (2019) Phytochemical analysis and total antioxidant capacity of rhizome, above-ground vegetative parts and flower of three Iris species. Chem Biodiversity16(3):e1800565

Krakhmal NV, Zavyalova MV, Denisov EV, Vtorushin SV, Perelmuter VM (2015) Cancer Invasion :Patterns and Mechanisms. Acta Naturae 7(2):17-28

Lee B, Moon KM, Lee BS, et al. (2017) Swertiajaponin inhibits skin pigmentation by dual mechanisms to suppress tyrosinase. Oncotarget 8(56):95530-95541

Liu KD, Yang WQ, Dai MZ ,et al. (2022) Phenolic constituents with anti-inflammatory and cytotoxic activities from the rhizomes of Iris domestica. Phytochemistry 203:113370

Merino-Casallo F, Gomez-Benito MJ, Hervas-Raluy S, Garcia-Aznar JM (2022) Unravelling cell migration: defining movement from the cell surface, Cell Adhesion & Migration, 16:1, 25-64

Michalak A, Krauze-Baranowska M, Migas P, Kawiak A, Kokotkiewicz A, Królicka A (2021) Iris pseudacorus as an easily accessible source of antibacterial and cytotoxic compounds. J Pharm Biomed Anal 195:113863

Mody H, Vaidya TR, Ait-Oudhia S (2023) In vitro to clinical translational pharmacokinetic/pharmacodynamic modeling of doxorubicin (DOX) and dexrazoxane (DEX) interactions: Safety assessment and optimization. Sci Rep 13(1):3100

Moraes DFC, de Mesquita LSS, do Amaral FMM, de Sousa Ribeiro MN, Malik S (2017) Anticancer drugs from plants biotechnology and production of anti-cancer compounds. Springer, p 121-142

Mykhailenko O, Lesyk R, Finiuk N, et al. (2020) In vitro anticancer activity screening of Iridaceae plant extracts.

Rashidi R, Montazeri A, Soukhtanloo M ,et al. (2024) Moraea sisyrinchium inhibits proliferation, cell cycle, and migration of cancerous cells, and decreases angiogenesis in chick chorioallantoic membrane. Iran J Basic Med Sci 27(1):57-65

Shaik BB, Katari NK, Jonnalagadda SB (2022) Role of natural products in developing novel anticancer agents: a perspective. Chem Biodivers 19(11):e202200535

Sheikh I, Sharma V, Tuli HS, et al. (2020) Cancer chemoprevention by flavonoids, dietary polyphenols and terpenoids.

Biointerface Res Appl Chem 11(1):8502-8537

Sulaiman CT, Balachandran I (2016) LC/MS characterization of antioxidant flavonoids from Tragia involucrata L. Beni-Suef University J Basic Appl Sci 5:231-235

Tan T, Li J, Luo R, et al. (2021) Recent advances in understanding the mechanisms of elemene in reversing drug resistance in tumor cells: a review. Molecules 26(19): 5792

Thelen P, Seseke F, Ringert RH, Wuttke W, Seidlová-Wuttke D (2006) Pharmacological potential of phytoestrogens in the treatment of prostate cancer. Urologe A 45(2):195-6, 197-201

Trejo-Solís C, Serrano-Garcia N ,Escamilla-Ramírez Á, et al. (2018) Autophagic and apoptotic pathways as targets for chemotherapy in glioblastoma. Int J Mol Sci 19(12)

Tuyiringire N, Deyno S, Weisheit A, et al. (2020) Three promising antimycobacterial medicinal plants reviewed as potential sources of drug hit candidates against multidrug-resistant tuberculosis. Tuberculosis (Edinb) 124:101987

Wang LJ, Shi HR (2020) Proliferation and invasion of ovarian cancer SK-OV-3 cells inhibited by Tectoridin. Zhonghua Zhong Liu Za Zhi 42(7):570-574

Wang X, Zhang C, Bao N (2023) Molecular mechanism of palmitic acid and its derivatives in tumor progression. Front Oncol 13:1224125

Wani SH, Padder BA, Mokhdomi T, et al. (2017) Antiproliferative activity of methanolic extracts of different Iris plant species against A549 and Caco-2 cell lines. J Pharmacogn Phytochem 6:1034-1037

Xiong L, Guo W, Yang Y, et al. (2021) Tectoridin inhibits the progression of colon cancer through downregulating PKC/p38 MAPK pathway. Mol Cell Biochem 476(7):2729-2738

Xu J, Sun S, Zhang W, et al. (2022) Irigenin inhibits glioblastoma progression through suppressing YAP/β-catenin signaling. Front Pharmacol 13:1027577

Yazgan B, Ozcelik O, Ayar A, Renda G, Yıldırım T (2022) Cytotoxic and apoptotic effect of Iris taochia plant extracts on human breast cancer (MCF-7) cells. Current Proteomics 19(1):91-101

Yeh LT, Hsu LS, Chung YH, Chen CJ (2020) Tectorigenin inhibits glioblastoma proliferation by G0/G1 cell cycle arrest. Medicina (Kaunas) 56(12):681

Zeng L, Yuan S, Shen J, Wu M, Pan L, Kong X (2018) Suppression of human breast cancer cells by tectorigenin through downregulation of matrix metalloproteinases and MAPK signaling in vitro. Mol Med Rep 17(3):3935-3943