Black Sea Journal of Health Science
doi: 10.19127/bshealthscience.1100668

Open Access Journal
e-ISSN: 2619 – 9041

Research Article
Volume 5 - Issue 3: 395-403 / September 2022

# THE IMPACT OF ENDEMIC *IRIS TAOCHIA* ETHANOLIC EXTRACTS ON HUMAN LUNG ADENOCARCINOMA CELLS

**Nezahat KANDEMİR[1]*, Emine ÇELİKOĞLU[2], Şevket KANDEMİR[1], Umut ÇELİKOĞLU[3], Önder İDİL[4], Canan Vejselova SEZER[5], Hatice Mehtap KUTLU[5]**

[1]*Amasya University, Education Faculty, Department of Mathematics and Science Education, 05100, Amasya, Türkiye*
[2]*Amasya University, Faculty of Science, Department of Biology, 05000, Amasya, Türkiye*
[3]*Amasya University, Faculty of Science, Department of Chemistry, 05000, Amasya, Türkiye*
[4]*Amasya University, Education Faculty, Department of Basic Education, 05100, Amasya, Türkiye*
[5]*EskişehirTechnical University, Faculty of Science, Department of Biology, 26000, Eskişehir, Türkiye*

**Abstract:** *Iris taochia* is an elegant endemic plant in Türkiye and it has limited distribution. In this study, cytotoxic effects of ethanolic extracts from different parts and concentrations extracted from *I. taochia* collected from the surroundings of Tortum (Erzurum), on A549 human lung adenocarcinoma cell line were investigated. Cytotoxicity of extracts were evaluated by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) method. Apopototic activity of $IC_{50}$ values of extracts were evaluated with Annexin V and Caspase 3/7 assays. Ultrastructural changes of $IC_{50}$ doses treated cells were investigated by transmission electron microscopy. As a result, it was determined that ethanol extract of *I. taochia* showed significant cytotoxic activity on A549 cells after 24 hours the extract a dose-dependent reduction in cell viability. $IC_{50}$ values of above and below ground parts ethanolic extracts were determined as 7 µg/ml and 20 µg/ml respectively. Specifically, apoptosis inducing effect was increased at 7 and 20 µg/ml concentrations by 24 hours. We found that *I. taochia* ethanol extracts had antiproliferative and apoptotic effects on the human lung adenocarcinoma cells A549. However, further studies at molecular level are required to support our findings and to elucidate chemoproteventive and chemotherapeutic effects of *I. taochia* on lung cancer.

**Keywords:** *Iris taochia*, Endemic, Biological activity, Lung cancer, Apoptosis

***Corresponding author:** Amasya University, Education Faculty, Department of Mathematics and Science Education, 05100, Amasya, Türkiye
**E mail:** nezahatkndmr@gmail.com (N. KANDEMİR)

Nezahat KANDEMİR https://orcid.org/0000-0002-5428-4139
Emine ÇELİKOĞLU https://orcid.org/0000-0002-5956-0008
Şevket KANDEMİR https://orcid.org/0000-0001-6781-0057
Umut ÇELİKOĞLU https://orcid.org/0000-0003-0995-8154
Önder İDİL https://orcid.org/0000-0003-1744-4006
Canan Vejselova SEZER https://orcid.org/0000-0002-3792-5993
Hatice Mehtap KUTLU https://orcid.org/0000-0002-8816-1487

**Received:** April 08, 2022
**Accepted:** April 28, 2022
**Published:** September 01, 2022

**Cite as:** Kandemir N, Çelikoğlu E, Kandemir Ş, Çelikoğlu U, İdil Ö, Sezer CV, Kutlu HM. 2022. The impact of endemic *iris taochia* ethanolic extracts on human lung adenocarcinoma cells. BSJ Health Sci, 5(3): 395-403.

## 1. Introduction

Türkiye, with its different climatic and ecological conditions is one of the richest countries of the world in terms of plant species. Many plant species consumed for medical purposes are members of this rich flora (Bayram et al., 2010). A good number these plants are reported to have anticancer, antiulcer, antimicrobial, antioxidant and antifungal effects (Rigona et al., 2007; Conforti et al., 2009; Ertürk et al., 2010; Bhalodia and Shukla, 2011; Hacibekiroglu and Kolak, 2011; Kandemir et al., 2022). The resistance towards chemotherapotics that has developed in recent years rapidly has caused a pursuit of new active substances and the discovery of new and more effective active pharmeochological ingredients from plants has gained importance.

*Iris* L. genus has wide distribution in the Northern Hemisphere. In Türkiye, it is one of the richest genera in terms of number of species. *Iris* genus is represented with 50 taxa in Türkiye (Güner et al., 2012). The species of this genus, among flowering plants are mainly used as ornamental plants due to their colorful, showy and pleasant fragrant flowers (Kandemir and Yakupoğlu, 2016). *Iris* taxa contains many biologically active substances as alkaloids, saponins, tannins, steroids, isoflavonoids, flavanoids, flavones, iridal type triterpenoids and their glycosides, benzoquinones, flavones, c-glycosylxanthones, glycosylflavones, phenolics, stilbene glycosides and cardiac glycosides (Wang et al., 2003; Nighat et al., 2008; Ma et al., 2012; Tantry et al., 2013; Kassak, 2014; Kukula-Koch et al., 2015). Isoflavonoids, flavanoids, quinones and xanthones of these are common substances in *Iris* species (Orhan et al., 2003; Asghar et al., 2010; Kassak, 2012). While the isoflavonoids are found in the rhizomes, c-glycosylxanthones, xanthone glycoides and flavonoid aglycones are found in the leaves and flowers of *Iris* taxa

(Kassak, 2012). *Iris* taxa have been used as medicine (in the treatment of cancer, inflammation, bacterial and viral infections and venereal disease) (Wollenweber et al., 2003; Fang et al., 2008; Conforti et al., 2009; Sabrin et al., 2012; Xie et al., 2013; Bozyel et al., 2019; Yazgan et al., 2022) and as economic plants (in perfumes and cosmetics) since the very early years due to the piscicidal, antineoplastic, antioxidant, antitumor, antiplasmodial, antiulcer, molluscicidal, estrogenic, hypolipidemic and anti-tuberculosis properties (Bonfils et al., 2001; Wang et al., 2003; Orhan et al., 2003; Rigano et al., 2009; Fang et al., 2008; Conforti et al., 2009; Huwaitat et al., 2013) of isolated secondary metabolites.

Cancer, as a major health problem, is defined as uncontrolled cell proliferation and spread. Depending on the stages of cancerous cells, surgical interventions, radiotherapy, chemotherapy, immunotherapy or hormone replacement therapies are among the options. In general, chemotherapeutic drugs affect apoptotic cell death and have a cytotoxicity effect on cancerous cells (Fang et al., 2008; Yazgan et al., 2022). For this reason, finding anti-cancer agents that are more effective on cancer cells as soon as possible increases its importance day by day. On the other hand, appotosis is programmed cell death, an important molecular mechanism that removes abnormal and damaged cells. Studies have reported that it is necessary to develop innovative methods for cancer treatment and to recognize molecular targets in apoptotic cell death pathways (Huang et al., 2012). In this regard, the use of herbal drugs for therapeutic purposes is increasing day by day. It has been reported that there are 160 plant taxa and 17 multi-herbal formulas used in cancer treatment in Turkish traditional medicine today (Bozyel et al., 2019).

*Iris taochia* Woronow ex Grossh. belongs to subgenus *Iris* of *Iris* genus. This subgenus is represented by 8 taxa in Türkiye Flora 4 of which *(I. juaonia* Schott &Kotschy ex Schott*., I. taochia, I. schachtii* Markgraf and *I. purpureobractea* B. Mathew & T. Baytop) are endemic to Türkiye. *I. taochia*, endemic Irano-Turanien element, is distributed only in the North East Anatolia (Erzurum-Tortum) in Türkiye. It is a rhizomatous and perennial plant and its length is 18.5-30 cm (Figure 1). This species has yellow and purple colored, sweet-smelling and showy 2-5 flowers (Mathew, 1984; Kandemir, 2006). *I. taochia* is used as a decorative plant because of the above mentioned characteristics and is called "Tortum suseni" in Türkiye. It contains iridals and essential oils in the rhizomes. According to International Union for Conservation of Nature (IUCN) endangered categories, this taxa are in the VU (Vulnerable) category in Türkiye (Ekim et al., 2000).

Based on the fact that there has been no recorded study on this endemic species, here it is aimed to investigate the impact of ethanolic *I. taochia* extracts on human lung adenocarcinoma (A549) cells mainly focusing on potent cytotoxic, antiproliferative and apoptosis triggering activities.

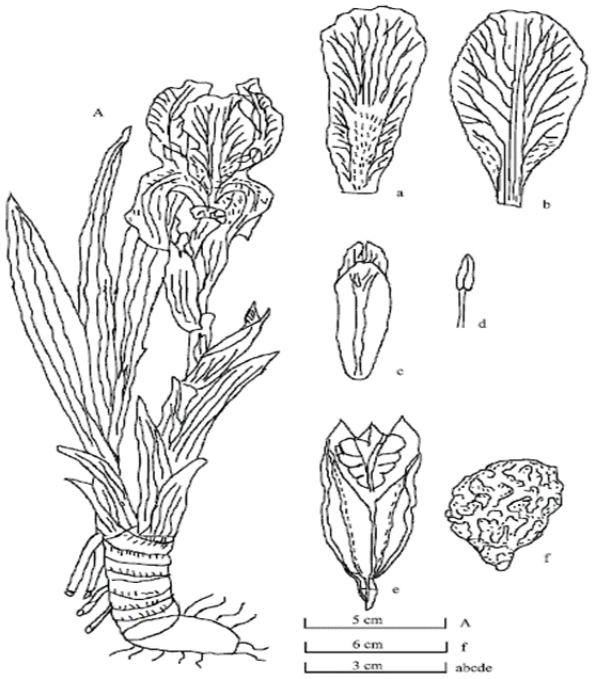


**Figure 1.** General appearance of *Iris taochia*. A= Plant, a=Outer tepal, b=Inner tepal, c=Style, d=Stamen, e=Capsule, f=Seed (illustrated by N. Kandemir from the living specimen flowered).

## 2. Materials and Methods

### 2.1. Preparation of Plant Materials

The samples of *I. taochia* were collected from vicinity of Tortum (Erzurum) during the flowering period in July 2018 (Figure 2). Taxonomic description of the species was made according to Mathew (1984). The locality of collected plant samples is given below; A8 Erzurum: Between Tortum and Oltu (2 km from Tortum), rocky areas, 1650 m., 20 July 2018, Kandemir, 867. A8 Erzurum: Near Tortum, rocky areas, 1500 m., 20 July 2018, Kandemir, 869.

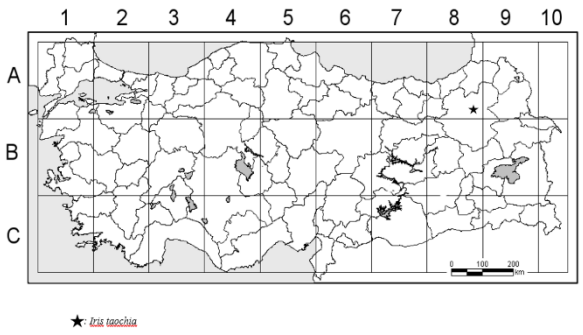


**Figure 2.** The distribution areas of *Iris taochia* in Türkiye

### 2.2. Preparation of Ethanolic Extracts from Plant Materials

The below-ground (rhizome and root) and above-ground parts (scape, leaf, flower, fruit and seed) of plant samples were cleaned, cut into pieces and dried on the benches at room temperature and in shadow in laboratory

conditions. They were often stuffed to prevent molding. Then, dried samples were grinded in the mill with sieve 2 mm and speed 500 rpm. Dried plant samples were stored in cloth bags. Plant powder was extracted with ethanol by maceration. Plant powder and ethanol (1:20 m/V) were continuously stirred at room temperature in dark for 72 hours (Trusheva et al., 2007). Then, extracts were filtered through 0.22 µm pore size membrane filter and stored at 4°C. Ethanol was evaporated in room temperature for measuring crude weight of extracts.

**2.3. Evaluating the Cytotoxicity of the Extracts by MTT Assay**

Different concentrations (ranging from 1.56-100 µg/ml) of the ethanolic extracts were prepared by dilution method in 96-well plates with fresh prepared growth medium. A549, human lung adenocarcinoma cells were seeded $2x10^3$ cells per well in the 96-well plate containing the above given concentrations. Cells were incubated for 24 hours at 37°C in a humidifed atmosphere of 5% $CO_2$ in air. After the incubation period 20 µl of MTT solution (5 mg/ml) was added per well and allowed to incubations in the same conditions for 2 hours. At the end of incubation period the growth media were changed with 200 µl/well of dimethyl sulphoxide and samples were kept at room temperature for 5 minutes. Samples were prepared in triplicates and absorbances were read on an ELISA reader at a wavelength of 570 nm (n = 3) (Mosmann, 1983). Then $IC_{50}$ concentrations were determined from the obtained viability percentages calculated with the following equation 1 (Edmondson et al., 1988).

Cell proliferation= [OD sample] × 100 / [OD control] (1)

Where; OD is optical density

**2.4. Annexin-V Analysis**

The apoptosis triggering action of *I. taochia* extract on A549 cells was evaluated by Annexin-V FITC/propidium iodide (PI) staining. For this manner, the cells were incubated with the $IC_{50}$ value of plant extracts for 24 hours. At the end of incubation period, 100 ml of untreated and treated cells were transferred to separate tubes. 100 µl of Annexin-V reagent was added to each tube and allowed to incubation in dark, for 20 minutes at room temperature. After the incubation period, samples were analyzed on Muse™ Cell Analyzer (Merck, Millipore, Hayward, California, USA). All the samples were prepared in triplicate according to the user manual of Muse® Annexin-V and Dead Cell Assay Kit.

**2.5. Caspase 3/7 Analysis**

A549 cells treated with $IC_{50}$ value of plant extract for 24 hours and untreated A549 cells were prepared for incubation by adding 5 µl Muse® Caspase 3/7 working solution (1:8 in 1 X PBS) to 50 µl of the cells. After the incubation period, 150 µl of 7-AAD working solution (2µl of 7-AAD to 148 µl of 1X assay buffer) was added. At the end of the incubation all samples were analyzed on Muse™ Cell Analyzer (Merck, Millipore, Hayward, California, USA). All the samples were prepared in triplicate according to the user manual of Muse® Caspase 3/7 Assay Kit.

**2.6. Transmission Electron Microscopy for Analyzing the Ultrastructural Changes**

The test cells treated with $IC_{50}$ concentration of the *I. taochia* extracts for 24 hours were fixed in glutaraldehyde and post was fixed in osmium tetraoxide. Following the fixation, the cells were dehydrated in graded ethyl alcohol and embedded in Epon 812 epoxy. Obtained blocks were sectioned on ultramicrotome. Thin sections were prepared by using a glass knife of a maximum thickness of 100 nm and stained in lead citrate and uranyl acetate. Stained samples were observed under a TEM (FEI Tecnai BioTWIN, Limmen, The Nederland) (Vejselova and Kutlu, 2015).

**2.7. Statistical Analysis**

Statistical comparison of the samples was carried out by one-way analysis of variance for multiple comparisons using Graphpad Prism 7.0 for Windows. The data was expressed as means ± SDs.

## 3. Results

**3.1. MTT Assay Results**

Cytotoxicity activity of *I. taochia* below and above ground parts ethanolic extracts were carried out against A549 cell line at different concentrations to determine the $IC_{50}$ (50% growth inhibition) by MTT assay. MTT assay of *I. taochia* showed significant effect on A549 in concentration range between 1,56 to 100 µg/ml compared with control. $IC_{50}$ value of below ground parts were detected to be 20 µg/ml for 24 hours (Figure 3). $IC_{50}$ value of above ground parts were detected to be 7 µg/ml for 24 hours (Figure 4).

**3.2. Annexin V Staining Results**

To evaluate the apoptosis level, annexin-V antibody was used. Annexin V staining results of untreated A549 cells (Figure 5A) showed 90.40% live, 1.25% early apoptotic and 1.30% late apoptotic cells. In A549 cells treated with $IC_{50}$ value of ethanolic extract of *I. taochia* above ground parts for 24 hours (Figure 5B) percentage of live cells were detected to be 86.10; whereas, 6.70% of these cells were in early apoptotic and 1.75% in late apoptotic stage. Live cell percentages in A549 cells treated with $IC_{50}$ value of ethanolic extract of *I. taochia* below ground parts (Figure 5C) for 24 hours were 7.10 and 0.25 in early apoptosis and 10.20 in late apoptosis.

**3.3. Caspase 3/7 Analysis Results**

In apoptosis profile of untreated A549 cells (Figure 6A) percentages of live cells detected to be 98.61%. Apoptotic/dead cells were 1.20% in the same group and 0.14% of cells were apoptotic. Only 0.05% of the control A549 cells were dead. In A549 cells treated with $IC_{50}$ concentration of *I. taochia* below ground parts ethanolic extract for 24 hours (Figure 6B) the percentage of live cells was 79.64%. The percentage of apoptotic/dead cells in this group was 18.84%, 0.97% were apoptotic and

0.55% were dead. In the A549 cells treated with $IC_{50}$ concentration of *I. taochia* below ground parts ethanolic extract (Figure 6C) for 24 hours' percentage of live cells was detected to be 28.59. Of these cells 1.48% were dead and 69.21% apoptotic/dead and 0.72% apoptotic.

**3.4. Ultrastructural Changes Detected by Transmission Electron Microscopy**

In order to determine whether the growth inhibition by plant extracts were associated with apoptosis, we further examined the morphological changes A549 cancer cell lines under transmission electron microscope. The control cells demostrated fusiform cell shape and cantact cell membrane. While A549 cells that were treated with the $IC_{50}$ value of *I. taochia* above ground parts ethanolic extract for 24 hours were displaying a circular cell shape, chromatin condensation, nuclear membrane disintegration, holes on the cytoskeleton, swelling in the endoplasmic reticulum tubes, loss of mitochondrial cristae and swelling of mitochondria and ondulation in nuclear membrane (Figures 7A, B, C and D); $IC_{50}$ value of *I. taochia* below ground parts ethanolic extract in the same incubation time displayed in addititon to the circular cell shape and chromatin condensation, fragmentation of nucleus, blebbings on cell membrane, disintegration of nuclear membrane and loss of mitochondria were significantly determined at the micrographs (Figures 7E and F).

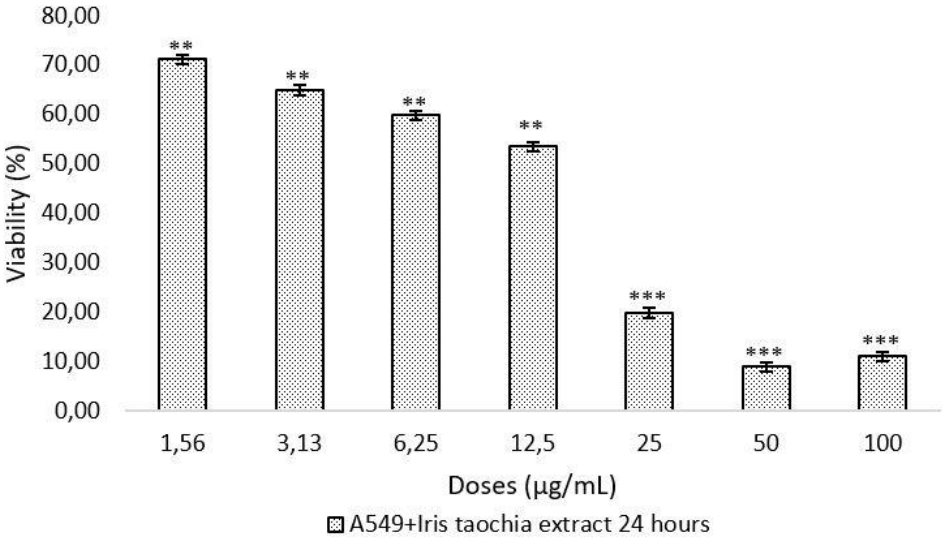


**Figure 3.** Viability percentages of A549 cells treated with ethanolic extract of *Iris taochia* below ground parts for 24 hours. ** P<0.01; *** P<0.05

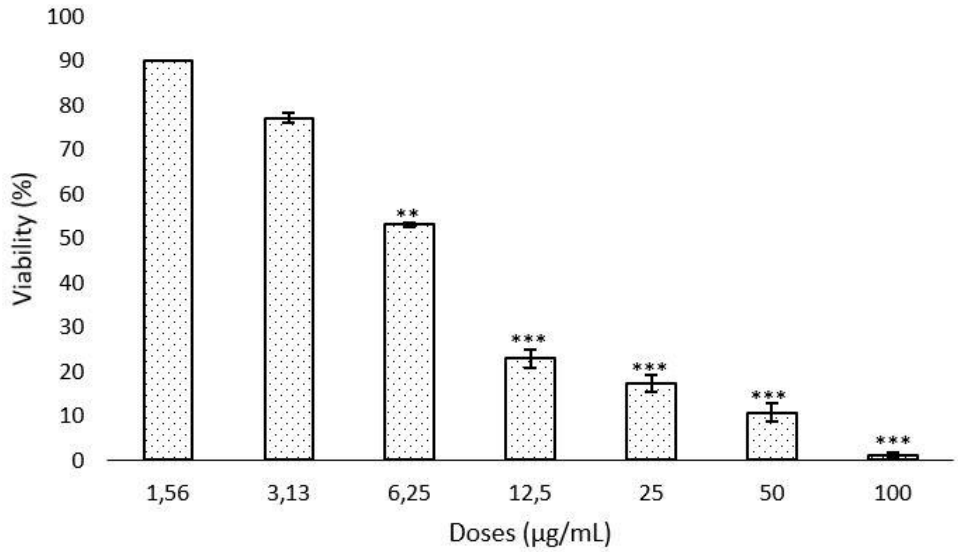


**Figure 4.** Viability percentages of A549 cells treated with ethanolic extract of *Iris taochia* above ground parts for 24 hours. ** P<0.01; *** P<0.05

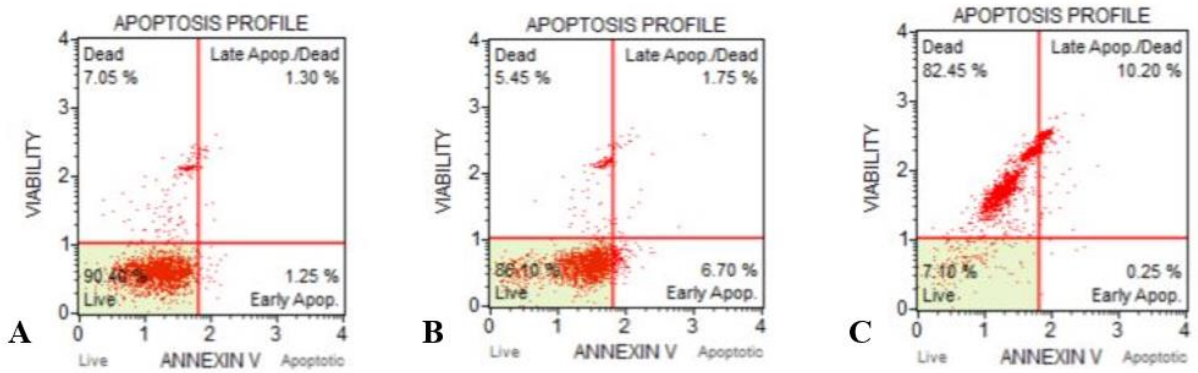


**Figure 5.** Percentages of apoptotic A549 cells treated with $IC_{50}$ values of *Iris taochia* extracts for 24 hours. A= Untreated A549 cells, B= $IC_{50}$ values of *Iris taochia* above ground parts, C= $IC_{50}$ values of *Iris taochia* below ground parts.

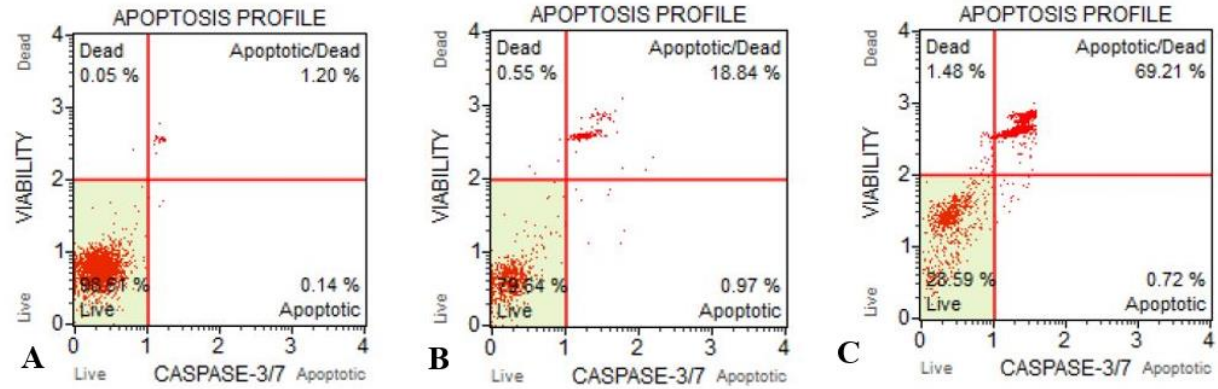


**Figure 6.** Apoptosis profiles of untreated A549 cells and A549 cells treated with $IC_{50}$ values of *Iris taochia* extracts for 24 hours. A= Untreated A549 cells, B= $IC_{50}$ values of *Iris taochia* above ground parts, C= $IC_{50}$ values of *Iris taochia* below ground parts.

## 4. Discussion

Lung cancer has become one of the common diseases worldwide that in terms of the cause of death of the patients (Duan and Zhang, 2006). Chemotherapy is an effective approach to cancer therapy including lung cancer, but it is limited since the developing resistance towards current chemotherapeutics like vinblastine and paclitaxel has been reported frequently (Spitz et al., 2009; Hsieh et al., 2010; Obradovic et al., 2013). This fact indicates a need for novel chemotherapy agents that are effective in low concentrations and short time application. Recently, plant-derived drugs have been made and become a good alternative in cancer therapy (Balunas and Kinghorn, 2005). Consequently, this research is focused on the investigation of anticancer activity of *I. taochia*, which belongs to the largest genus in the Iridaceae family, extracts on the A549, human lung adenocarcinoma cell line.

Our MTT assay findings (Figures 3 and 4) showed that ethanolic extract of *I. taochia* below ground parts remarkably decreased the viability of the A549 cells in concentration-dependent manner in short term application of 24 hours. The determined $IC_{50}$ value of the extract was to be 20 µg/ml. Viability percentages determined from the A549 cells treated with ethanolic extract of *I. taochia* above ground parts (Figure 3) significantly decreased with the increase of the concentration of the applied extract in the same incubation period. The $IC_{50}$ value for this extract was detected to be 7 µg/ml. These values can be interpreted as effective in the inhibition of the proliferation of A549 cells at low doses and in short-time application, both for the extracts from above and below and ground parts of *I. taochia*. Similarly, in a research the anticancer activity of an Iridaceae family member *Romulea tempskyana* extract on hepatoma G2 and H1299 cell lines is showed. In 24 hours of application of $IC_{50}$ value of the extract for G2 cells was reported as 94.79 µg/ml, whereas; it was 76.15 µg/ml for H1299 cells in the same time of incubation (Ozkan and Erdogan, 2012). Additionally, growth of 25.2% of the treated large lung carcinoma cells was reported to be inhibitted in 48 hours' application of *Iris pseudopumila* Tineo extracts obtained from the flowers of the species at the highest concentration, 100 µg/ml.

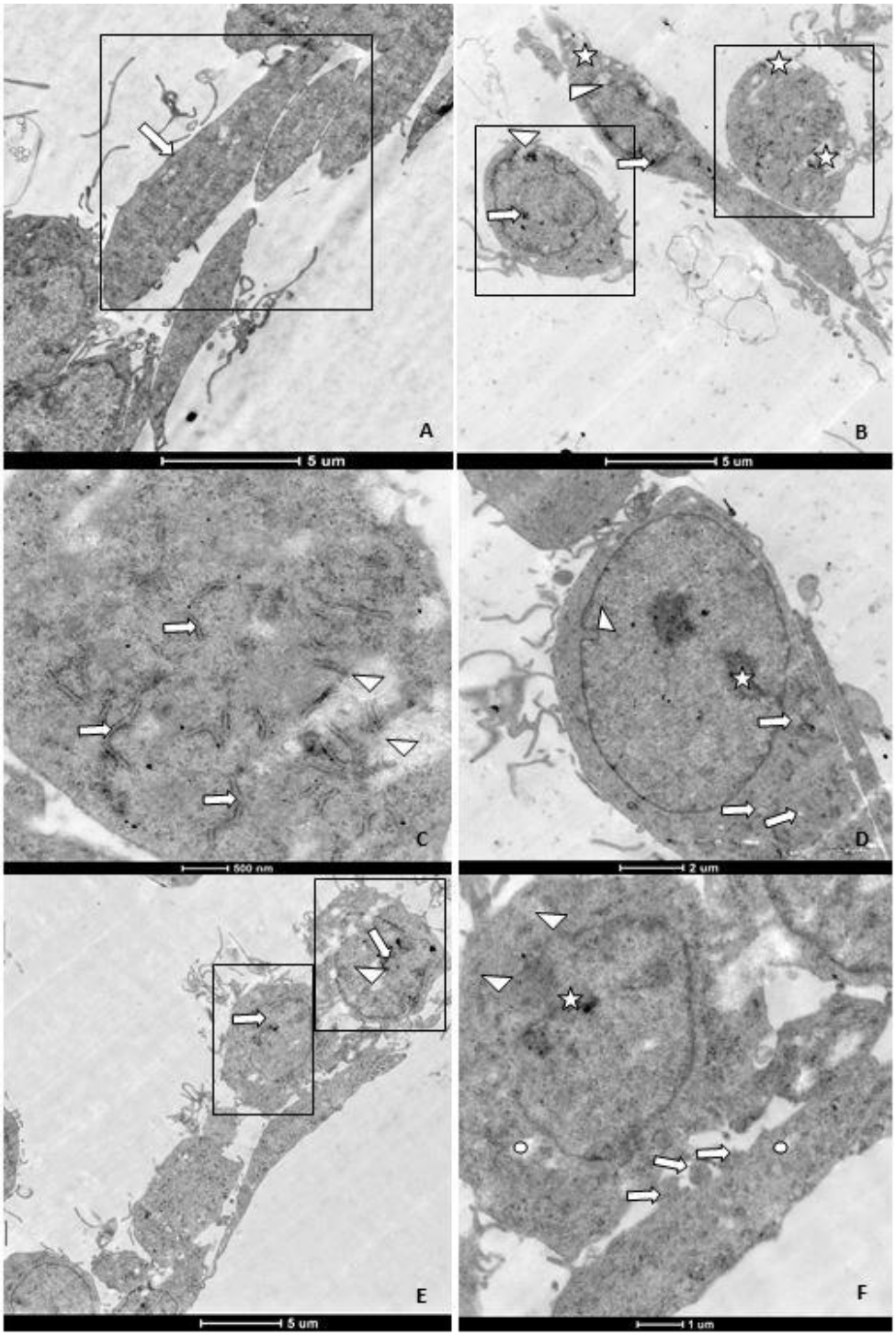


**Figure 7.** Ultrastructure of the untreated A549 cells. A (6000×)= □ fusiform cell shape, ⇨ compact cell membrane. A549 cells treated with the $IC_{50}$ value of *Iris taochia* above ground parts ethanolic extract for 24 hours, B (6000×)= □ circular cell shape, ⇨ chromatin condensation, ▽ nuclear membrane disintegration, ☆ holes on the cytoskeleton, C (11,500×)= swelling in the endoplasmic reticulum tubes, ▽ loss of mitochondrial cristae and swelling of mitochondria, D (8200×)= chromatin condensation, ⇨ loss of mitochondrial integrity, ▽ ondulation in nuclear membrane. A549 cells exposed to $IC_{50}$ concentration of *Iris taochia* below ground parts ethanolic extract for 24 hours, E (6000×)= □ circular cell shape, ⇨ chromatin condensation, ▽ fragmentation of nucleus, F (8200×)= ⇨ blebbings on cell membrane, ☆ chromatin condensation, ▽ disintegration of nuclear membrane, ○ loss of mitochondria.

The percentage of inhibited cells at *I. pseudopumila* extract obtained from the rhizomes of the species was detected to be 31.5 (Conforti et al., 2009). Our results were found to be very low when compared to the above mentioned findings. This might be attributed to the difference of the used plant species, consequently, the content of the extracts as well as the type of the exposed cell lines. In previous pharmacological studies it was

reported that pharmacological activity of *Iris* species is mainly attributed to their flavonoids ingredients (Fang et al., 2008). Consequently, this might be occured in the antiproliferative action of the *I. taochia* extracts in our study but this fact needs to be further investigated to be confirmed. Additionally, since the active ingredients of many drugs used in cancer treatment today are obtained from medicinal plants, anticancer activities of medicinal plants should be screened for further use (Bozyel et al., 2019). On the other hand, when the anticancer properties of medicinal plants were examined, it was determined that the active substances in medicinal plants were effective on different types of cancer such as prostate, colon, stomach breast and leukemia (Padmaharish and Lakshmi, 2017).

Apoptosis is one of the main regulatory pathways in proliferation and death of cells. This programmed cell death occurs as a response to initiating intracellular and/or extracellular signals. Characteristic physiological changes of apoptosis were well described of which externalization of phosphatidylserine to the cell surface can be showed by annexin-V that is a calcium-dependent phospholipid-binding protein that binds the externalized phosphatidylserine during apoptosis. Also it may refer to the membrane disintegration in early apoptotic cells (Kerr et al., 1972; Wyllie et al., 1980; Wyllie, 1993; Majno and Joris, 1995; Rudin and Thompson, 1997). The induction of apoptosis in cancer cells has been a common investigation issue, recently (Ghobrial et al., 2005). As it is shown in our annexin V staining results, in A549 cells treated with $IC_{50}$ value of ethanolic extract of *I. taochia* above ground parts for 24 hours (Figure 5B); the percentage of live total apoptotic cells (8.45%) was low when compared to that of ethanolic extract of *I. taochia* below ground parts (10.45%) (Figure 5C). Extract of these species below ground parts can be considered as more effective in activation of apoptotic cell death but the difference between the action of extracts is very slight. Yazgan et al. (2022) examined how *I. taochia* extracts affect apoptotic activity on MCF7 cells and research results showed that MeOH extract of *Iris taochia* on MCF7 cells could be a regulator of cell death proteins, growth factors and cell repair mechanisms.

Proapoptotic signals trigger apoptotic cell death by activating specific cysteine proteases so called caspases. Some of these enzymes are initiators of intracellular event cascade and the others act further to direct cellular breakdown through cleavage of structural proteins. The latter group of caspases are caspase-3 and caspase-7 (Riedl and Shi, 2004; Benetti and Roizman, 2007). Activation of these caspases is a hallmark of apoptosis. In this study, activation of these caspases are measured in untreated A549 cells and cells treated with the *I. taochia* extracts. In untreated A549 cells (Figure 6A) percentages of live cells were detected to be 98.61%. That means caspases are not activated. In A549 cells treated with $IC_{50}$ concentration of *I. taochia* above ground parts extract for 24 hours (Figure 6B) the percentage of total apoptotic cells was 19.81%. In the A549 cells treated with $IC_{50}$ concentration of below ground parts extract of the plant (Figure 4C) 69.93% were apoptotic. Activation of caspases 3/7 was determined more than that of above ground parts extract applied cells. This may be as a result of the differences in the ingredients of the extracts.

Physiological changes that occur during apoptotic cell death can be used in evaluating the type of cell death. Many of these changes can be showed by transmission electron microscopic evaluation that is taken as 'gold standard' in determined the ultrastructural changes of cells. Cleavage and degradation of specific cellular proteins, fragmentation of nuclear chromatin, and loss of membrane integrity as well as the integrity of organels mainly mitochondria can be observed (Kerr et al., 1972). In a research by Özkan and Erdoğan (2013), the effects of the natural agents' eugenol, eucalyptol, terpinen-4-ol, and camphor on cell membrane and DNA damage were investigated in human lung cancer cell lines and they were reported to damage cell membrane and the DNA. Similarly, in our study the natural ethanolic extracts of *I. taochia* ground parts and above parts were investigated to understand their effect on the A549 cell ultrastructure in respect of finding the type of cell death. Ultrastructural changes determined on the A549 cell treated with the $IC_{50}$ concentration of *I. taochia* above ground parts extract for 24 hours were found to be circular cell shape, chromatin condensation, nuclear membrane disintegration, holes on the cytoskeleton, swelling in the endoplasmic reticulum tubes, loss of mitochondrial cristae and swelling of mitochondria and on dilation in nuclear membrane (Figures 7B, C and D). All TEM findings can be considered as significant signs of apoptosis. Especially loss of mitochondrial cristae and nuclear membrane disintegration indicate apoptotic cell death that further mechanism needs to be cleared with additional experiments. Comparatively, in A549 cells exposed to $IC_{50}$ value of *I. taochia* below ground parts extract for the same incubation time in addititon to the circular cell shape and chromatin condensation, fragmentation of nucleus, blebbings on cell membrane, disintegration of nuclear membrane and loss of mitochondria were significantly determined at the micrographs (Figure. 7E and F). Blebbing of the cell membrane and fragmentation of the nuclei are direct indicators of programmed cell death. This might be resulted from the higher $IC_{50}$ concentration determined than that of above ground parts extract for 24 hours. This finding is supported with our annexin V findings.

Taken all together, our results suggest that *I. taochia* ethanolic extracts both that from the above ground parts and below ground parts of the plant exibit their cytotoxicity as well as anticancer and apoptosis triggering activity at lower concentrations on the investigated human lung adenocarcinoma cells. These findings might be encouraging for sequential investigations in this field. Further research is required to unravel the deeper molecular mechanisms of action of

the above investigated extracts including with identification of the active compounds and their separate molecular targets for their effective usage in drug development processes for cancer therapy.

## 5. Conclusion

In conclusion, our experimental results can be considered as *I. taochia* ethanolic extracts are both obtained from the above and below ground parts of the plant showed growth-inhibiting, cytotoxic and apoptotic effects in the A549 human lung adenocarcinoma cell line.

**Author Contributions**

Concept: C.V.S. (%50) and H.M.K. (%50), Design: C.V.S. (%50) and H.M.K. (%50), Supervision: C.V.S. (%50) and H.M.K. (%50), Data collection and/or processing: E.Ç. (%50) and U.Ç. (50%), Data analysis and/or interpretation: C.V.S. (%50) and H.M.K. (%50), Literature search: C.V.S. (%25), H.M.K. (%25), N.K. (%25) and Ö.İ. (%25), Writing: C.V.S. (%25), H.M.K. (%25), N.K. (%25) and Ö.İ. (%25), Critical review: N.K. (%100), Submission and revision C.V.S. (%50) and H.M.K. (%50). All authors reviewed and approved final version of the manuscript.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Approval/Informed Consent**

Ethics committee approval was not required for this study because of there is no animal or human study. In this research, since artificial cell cultures were used, no ethical statement was needed.

## References


Asghar SF, Rehman HU, Rahman AU, Choudhary, MI. 2010. Phytochemical investigations on *Iris germanica*. Nat Prod Res, 24: 131-139.

Balunas MJ, Kinghorn AD. 2005. Drug discovery from medicinal plants. Life Sci, 78: 431-441.

Bayram E, Kırıcı E, Tansi S, Yılmaz G, Arabacı O, Kızıl S, Telci D. 2010. Possibilities of Increasing Production of Medical and Aromatic Plants. In: Turkey Agricultural Engineering Technical Conference Proceedings Book-I, January 11-15, Ankara, Türkiye, pp. 437-457.

Benetti L, Roizman B. 2007. In transduced cells, the US3 protein kinase of herpes simplex virus 1 recludes activation and induction of apoptosis by transfected procaspase 3. J Virol, 81: 10242-10248.

Bhalodia NR, Shukla VJ. 2011. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant. J Adv Pharm Technol Res, 2:104-109.

Bonfils JP, Pinguet F, Culine S, Sauvaire Y. 2001. Cytotoxicity of iridals, triterpenoids from *Iris*, on human tumor cell lines A2780 and K562. Planta Med, 67:79-81.

Bozyel ME, Bozyel EM, Canlı K, Altuner EM. 2019. Anticancer Uses of Medicinal Plants in Turkish Medicine. KSU J Agric Nat, 22: 465-484.

Conforti F, Menichini F, Rigano D, Senatore F. 2009. Antiproliferative activity on Human cancer cell lines after treatment with polyphenolic compounds isolated from *Iris pseudopumila* flowers and rhizomes. Z Naturforsch, 64:490-494.

Duan W, Zhang L. 2006. Cyclooxygenase inhibitors not inhibit resting lung cancer A549 cell proliferation. PLEFA, 74:317-321.

Edmondson JM, Armstrong LS, Martinez AO. 1988. A rapid and simple MTT-based spectrophotometric assay for determining drug sensitivity in monolayer cultures. Meth Cell Sci, 11:15-17.

Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z, Adıgüzel N. 2000. Endemics. In: Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z, Adıgüzel N, editors. Red Data of Turkish Plants, Nature Conservation Association Publications, Ankara, Turkey, pp 96.

Ertürk R, Çelik C, Kaygusuz R, Aydın H. 2010. Antimicrobial activities of commercial thyme and mint essential oils. CMJ, 32:281-286.

Fang R, Houghton PJ, Hylands PJ. 2008. Cytotoxic effects of compounds from *Iris tectorum* on human cancer cell lines. J Ethnopharmacol, 118:257-263.

Ghobrial IM, Witzig TE, Adjei AA. 2005. Targeting apoptosis pathways in cancer therapy. CA Cancer J Clin, 55:178-194.

Güner A. 2012. *Iris* L. In: Güner A, Aslan S, Ekim T, Vural M, Babaç MT, editors. Plants List of Turkey (Vascular Plants), Nezahat Gökyiğit Botanical Garden and Flora Research Association Publication İstanbul, Türkiye, pp 535-540.

Hacibekirglu I, Kolak U. 2011. Antioxidant and anticholinesterase constituents from the petroleum ether and chloroform extracts of *Iris suaveolens*. Phytother Res, 25: 522-529.

Hsieh CC, Hernández-Ledesma B, Ben O. 2010. Lunasin, a novel seed peptide, sensitizes human breast cancer MDA-MB-231 cells to aspirin-arrested cell cycle and induced apoptosis. Chem Biol Interact, 186: 127-134.

Huang KF, Zhang GD, Huang YQ, Diao, Y. 2012. Wogonin induces apoptosis and down-regulates survivin in human breast cancer MCF-7 cells by modulating PI3K-AKT pathway. Int. Immunopharmacol, 12:334-341.

Huwaitat S, Al-Khateeb E, Finjan S. 2013. Isolation and identification of some phytochemical compounds from different parts of *I. nigricans*. ESJ, 9:32-37.

Kandemir N. 2006. An investigation on the autecological of endemic *Iris taochia* Woronow ex Gross. (Iridaceae) distributed in the North East Anatolia Region. Pakistan J Biol Sci, 9:2753-2760.

Kandemir N, Yakupoğlu H. 2016. Morphological and anatomical properties of endemic *Iris nezahatiae* distributed in the North-East Anatolia region (Turkey). Bitlis Eren Univ J Sci Technol, 5:104-112.

Kandemir N, Kandemir Ş, Çelikoğlu E, Çelikoğlu U, İdil Ö. 2022. Antioxidant and antimicrobial activities of extracts obtained from different organs of *Heliotropium myosotoides* and *Heliotropium suaveolens* (Boraginaceae). BSJ Eng Sci, 5(3): https://doi.org/10.34248/bsengineering.1084135 (in press).

Kassak P. 2012. Secondary metabolites of the choosen genus *Iris* species. Acta Univ Agric et Silvic Mendel Brun, 60:269-280.

Kassak P. 2014. Screening of the chemical content of several *Limniris* group Irises. J Pharmacogn Phytochem, 3:11-14.

Kerr JF, Wyllie AH, Currie AR. 1972. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer, 26:239-257.

Kukula-Koch W, Sieniawska E, Widelski J, Urjin O, Głowniak P, Skalicka-Woźniak K. 2015. Major secondary metabolites of *Iris* spp. Phytochem Rev, 14:51-80.

Ma Y, Li H, Lin B. 2012. C-glycosylflavones from the leaves of *Iris tectorum* Maxim. Acta Pharm Sin B, 2:598-601.

Majno G, Joris I. 1995. Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol, 146:3-15.

Mathew B. 1984. *Iris* L. In: Davis PH, Mill RR, Tan K, editors. Flora of Turkey and the East Aegean Islands. Edinburg University, Edinburg, 8th, pp. 382-410.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival:application to proliferation and cytotoxicity assays. J Ummunol Methods, 65: 55-63.

Nighat N, Surrinder K, Mushtaq AQ. 2008. Isoflovonos from *I. kashmiriana.* J Asian Nat Prod Res, 10: 1137-1141.

Obradovic A, Zizic J, Trisovic N, Bozic B, Uscumlic G, Bozic B, Markovic S. 2013. Evaluation of antioxidative effects of twelve 3-substituted-5, 5-diphenylhydantoins on human colon cancer cell line HCT-116. Turk J Biol. 37:741-747.

Orhan I, Nasim S, Tener B, Ayanoglu F, Özgüven M, Choudhary IM, Rahman AU. 2003. Two isoflavones and bioactivity spectrum of the crude extract if *Iris germanica* rhizomes. Phytother Res, 17: 575-577.

Özkan A, Erdogan A. 2012. Antioxidant and anticancer activity of fresh corm extract from *Romulea tempskyana* (Iridaceae). Nat Prod Res, 26:2126-2128.

Özkan A, Erdoğan A. 2013. Membrane and DNA damaging/protective effects of eugenol, eucalyptol, terpinen-4-ol, and camphor at various concentrations on parental and drug-resistant H1299 cells. Turk J Biol, 37:405-413.

Padmaharish V, Lakshmi T. 2017. Anticancer Activities of Medicinal Plants –An Update. J Pharm Sci Res, 9: 432-440.

Riedl SJ, Shi Y. 2004. Molecular mechanisms of caspase regulation during apoptosis. Nat Rev Mol Cell Biol, 5:897-907.

Rigano D, Conforti F, Formisano C, Menichini F, Senatore F. 2009. Comparative free radical scavenging potential and cytotoxicity of different extracts from of *Iris pseudopumila* Tineo flowers and rhizomes. Nat Prod Res, 23:17-25.

Rudin CM, Thompson CB. 1997. Apoptosis and disease: regulation and clinical relevance of programmed cell death. Ann Rev Med, 48:267-281.

Sabrin RMI, Gamal AM, Nawal MAM. 2012. New Constituents from the rhizomes of Egyptian *Iris germanica* L. Molecules, 17:2587-2598.

Spitz G A, Furtado CM, Sola-Penna M, Zancan P. 2009. Acetylsalicylic acid and salicylic acid decrease tumor cell viability and glucose metabolism modulating 6-phosphofructo-1-kinase structure and activity. Biochem Pharmacol, 77:46-53.

Tantry MA, Ghazanfar K, Zargar UR. 2013. New alkylated benzoquinone from *Iris nepalensis.* Nat Prod Res, 27:1832-1836.

Trusheva B, Trunkova D, Bankova V. 2007. Different extraction methods of biologically active components from propolis: a preliminary study. Chem Cent J, 1:1-13.

Vejselova D, Kutlu HM. 2015. Inhibitory effects of salicylic acid on A549 human lung adenocarcinoma cell viability. Turk J Biol 39: 1-5.

Wang YQ, Tan JJ, Tan CH, Jiang SH, Zhu DY. 2003. Halophilols A and B, two new stilbenes from *Iris halophila*. Planta Med, 69:779-781.

Wollenweber E, Stevens JF, Klimo K, Kanauft J, Norbert F, Clarissa G. 2003. Cancer chemopreventive in vitro activities of isoflavones isolated from *Iris germanica*. Planta Med, 69:15-20.

Wyllie AH, Kerr JR, Currie AR. 1980. Cell death: the significance of apoptosis. Int Rev Cytol, 68:251-306.

Wyllie AH. 1993. Apoptosis (the 1992 Frank Rose memorial lecture). Br J Cancer, 67:205-208.

Xie X, Tian Y, Yin S, Lin Y, Tan G. 2013. Anticancer effects of *Ligusticum chuanxiong* Hort alcohol extracts on HS766 T cell. Afr J Tradit Complement Altern Med, 10:542-546.

Yazgan B, Özçelik Ö, Ayar A, Renda G, Yıldırım T. 2022. Cytotoxic and Apoptotic Effect of Iris taochia Plant Extracts on Human Breast Cancer (MCF-7) Cell. Curr Proteom, 19:91-101.