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Chemical Composition of Taraxacum Officinale Extracts and Anti-Proliferative Effect on A549 and HT-29 Cell Lines Through Regulating the Expression of Apoptosis-Related Genes

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ABSTRACT

The use of medicinal plants is still of great importance as an alternative treatment method in neurodegenerative diseases, cardiovascular patients and cancer. Taraxacum officinale plant is one of the medical plants used in the treatment of many diseases as diuretic, hypolipidemic and antidiabetic thanks to the active components it contains. In order to examine the effect of Taraxacum officinal on proliferation and apoptosis in cancer, in our study, we determined the chemical composition of the Taraxacum officinal plant by gas chromatography-mass spectrometry (GC-MS) and its antioxidant status using a commercial kit. Additionally, we determined the anti-cancer effect of water, methanol and chloroform extracts of the Taraxacum officinal plant on A549 and HT-29 cancer cell lines with the help of the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) method. Finally, we aimed to determine the relationship between the chloroform extract of the Taraxacum officinal plant, which has the highest anti-cancer properties, and apoptosis by determining the expressions of BAX, BCL-2 and Caspase-3, which are among the genes associated with apoptosis, in A549 and HT-29 cancer cell lines, with the help of Real-time Polymerase Chain Reaction (RT-PCR) analysis. We determined that the Taraxacum officinal plant has an anti-cancer effect by increasing caspase-3 expressions in A549 lung cancer cell lines and by increasing caspase-3 and BAX expressions and decreasing BCL-2 expression in HT-29 colon cancer cell lines. In light of the data we obtained, we can say that the Taraxacum officinal plant has anti-cancer properties that cause a decrease in cell proliferation by regulating the expression of genes related to apoptosis.

Keywords: Cancer, GC-MS, Taraxacum officinal, Apoptosis, Gene expression.

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Introduction

Medicinal plants have been used by humanity since ancient times to treat diseases through the active ingredients they contain [1]. Today, as a result of developments in the field of pharmacology, although non-plant-based synthetic drugs are produced for the treatment of diseases, active substances obtained from medicinal plants are still widely used [2]. In particular, the use of medicinal plants remains important as an alternative treatment method in neurodegenerative diseases, cardiovascular patients and cancer [3-5].

Taraxacum officinale plant is one of the medical plants used in the treatment of many diseases due to the active components it contains. Taraxacum officinale plant is a perennial 40 cm tall plant and grows in temperate and hot climates. It is a wild herbaceous plant that blooms in yellow and orange colors [6].

Considering the studies on the medical benefits of the Taraxacum officinale plant, it has been determined that the hydroethanolic extract of Taraxacum officinale has a diuretic effect on humans [7]. In another study, it was shown that water and methanol extracts of the plant inhibit the activities of α-amylase and α-glucosidase enzymes under in vitro conditions, thus having anti-diabetic effects [8]. When the root and leaf of Taraxacum officinale were included in the diet of rabbits fed with high cholesterol, it was determined that the activities of enzymes that produce antioxidant effects increased and lipid levels decreased in plasma samples taken from rabbits. Based on these results, it has been reported that Taraxacum officinale has antioxidant and hypolipidemic effects and may contribute to the treatment of cardiovascular diseases by providing protection against atherosclerosis [9]. However, it was determined that hepatoxicity in mice in which liver damage was caused by the administration of acetaminophen and in mice given extracts obtained from Taraxacum officinale leaves was significantly reduced through the antioxidant properties of Taraxacum officinale [10]. In studies on the role of Taraxacum officinal in cancer, it has been determined that it reduces cell proliferation in breast and prostate cancer [11], has an antitumor effect in pediatric cancers [12], and its hydroethanolic extract induces apoptosis in breast cancer cells [13].

The emergence of cancer is associated with a shift in the balance of proliferation and apoptosis, which is maintained in healthy cells, towards proliferation. Apoptosis is a programmed form of cell death in cells that has genetic basis [14]. There are many genes associated with apoptosis and the proteins these genes encode.

Caspase-3, BCL-2 and BAX are among the proteins that have the most important role in the regulation of apoptosis. While caspase-3 and BAX mediate the induction of apoptosis, BCL-2 plays a role in inhibiting it [15].

In order to examine the effect of Taraxacum officinal on proliferation and apoptosis in cancer, in our study, we aimed to determine the anticancer effect of water, methanol and chloroform extracts of the Taraxacum officinal plant, whose chemical composition and antioxidant status we analyzed, on A549 and HT-29 cancer cell lines, and to determine the anti-cancer effect of the Taraxacum officinal plant exposed to chloroform extract. We aimed to determine their relationship with apoptosis by determining the expressions of BAX, BCL-2 and Caspase-3 in A549 and HT-29 cancer cell lines.

Materials and Methods

Collection of Plant Material

Taraxacum officinal plant was collected in SİVAS province of Turkey in 2023 and stored in the dark compartment under room temperature conditions.

Extract Preparation

After the Taraxacum officinal plant was collected, it was divided into 10 gram portions. Then, 10 grams of plant samples were ground. Water, methanol and chloroform extracts were prepared from the ground plant samples. The methanol extract of the plant was prepared in the Soxhlet extractor under 60°C and 2-hour extraction conditions. The extract was concentrated using vacuum below 45°C. A portion of the extract, from which methanol was removed, was dissolved in chloroform and water. The resulting fractions were evaporated and the remaining part was stored in the dark at 4°C [16].

Gas Chromatography-mass Spectroscopic Analysis

Shimadzu GCMS-QP2010 Ultra Gas Chromatograph Mass Spectrometer (Shimadzu, Kyoto, Japan) device was used in GC-MS analyzes to determine the chemical content of the plant. The system setup includes a Flex 2 autosampler (EST Analytical, Ohio, United States) for automatic HS-SPME sampling. Data were analyzed with GC-MS Postrun Analysis software (ver. 4.53) [16].

Cell Culture Maintenance

Preparation of cell culture

A549 and HT-29 cells were grown in medium containing Dulbecco's Modified Eagle Medium (DMEM). The medium also contained 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin of the total volume. Cells were grown under 37°C and 5% CO2 culture conditions [17].

MTT method

Water, methanol and chloroform extracts of the Taraxacum officinal plant were applied to A549 and HT-29 cells for 24, 48 and 72 hours and IC50 values were determined via the MTT method. For this, cells were seeded in 96-well plates at 1 x 10^5/ml and incubated overnight. Then, the % viability values in each well were determined in accordance with the MTT kit protocol. % viability graphs were drawn with GraphPad Prism and statistical analysis was performed [16]. Images of A549 and HT-29 cells exposed to Taraxacum officinal plant extracts for 24 hours were recorded through a microscope.

Expression analysis of BAX, BCL-2 and Caspase-3, Genes Associated with Apoptosis

cDNA was synthesized using the kit (Thermo Scientific, USA) from total RNA isolated using the kit (GeneAll Hybrid-R, South Korea) from A549 and HT-29 cells exposed to the IC50 dose of chloroform extract of the Taraxacum officinal plant for 24 hours. Using these cDNAs, expression analysis of Caspase-3, BAX and BCL-2 was performed on the RT-PCR device with the help of the SYBR Green qPCR Mastermix kit [18]. Statistical analysis of the data was done with the program (https://geneglobe.qiagen.com/us/analyze).

Total Antioxidant Status (TAS) Measurement

The method developed by Erel was used to measure TAS levels in extracts via spectrophotometer. The basis of this method is based on the measurement of the inhibition of the amounts of colored dianisidyl radicals formed by free radical reactions in the Fenton reaction by antioxidants. The higher the active antioxidant capacity in the extracts, the more color formation is suppressed. In this method, the results are expressed as milimolar Trolox equivalents per L extract (mmol Trolox Equiv./L). In our study, TAS was determined by spectrophotometer using a commercial kit in accordance with the manufacturer's instructions (Rel Assay Diagnostic, Türkiye) [19,20].

Results

Chemical Composition of the Plant

The chemical composition was determined by GC-MS analysis of the chloroform extract of the Taraxacum officinal plant. It was determined that there are 38 active components in the chloroform extract of the Taraxacum officinal plant. In the chloroform extract of Taraxacum officinal plant.; Hexadecanoic acid, Phytol, 9-Octadecanamide, Neophytadiene, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione were determined to be the most abundant active compounds at the concentration of 16.25%, 14.39%, 11.02%, 9.42% and 5.07%, respectively.

MTT Assay

A549 and HT-29 cell lines were exposed to 1000µg/ml, 500µg/ml, 250µg/ml, 100µg/ml, 50µg/ml and 10µg/ml chloroform, methanol, and water extracts of Taraxacum officinal plant for 24, 48, and 72h to determine IC50 effective doses (Figures 1-3).

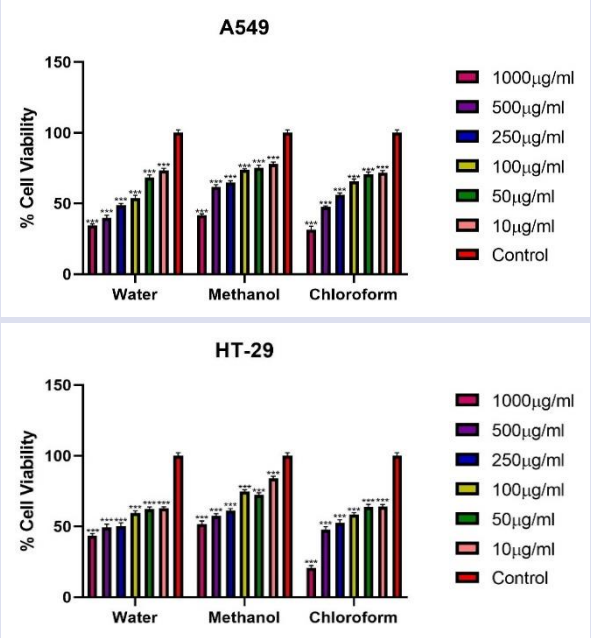


Figure 1. Viability percentages of A549 and HT-29 cells after 24 h exposure (***P < 0.001, **P < 0.01, *P < 0.05).

In A549 cell lines, the IC50 effective dose of the water extract was determined as 195.30 µg/ml, the IC50 effective dose of the methanol extract was 977.90 µg/ml, and the IC50 effective dose of the chloroform extract was 322.20 µg/ml for the 24th hour. In HT-29 cell lines, the IC50 effective dose of water extract at the 24th hour was determined as 379.20 µg/ml, the IC50 effective dose of methanol extract was 1208.00 µg/ml, and the IC50 effective dose of chloroform extract was 168.60 µg/ml (Figure 1.).

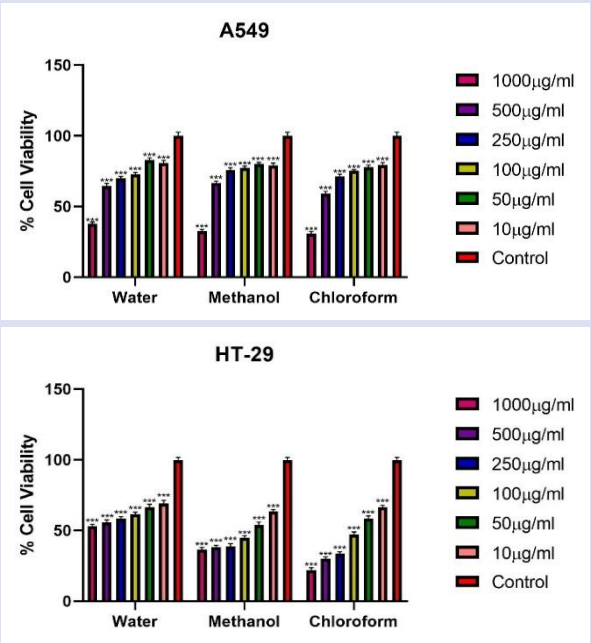


Figure 2. Viability percentages of A549 and HT-29 cells after 48h exposure (***P < 0.001, **P < 0.01, *P < 0.05).

In A549 cell lines, the IC50 effective dose of water extract was determined as 853.00 µg/ml, the IC50 effective dose of methanol extract was 813.60 µg/ml, and the IC50 effective dose of chloroform extract was 604.50 µg/ml for the 48th hour. The IC50 effective dose of the water extract was 853.00 µg/ml, the IC50 effective dose of the methanol extract was 813.60 µg/ml, and the IC50 effective dose of the chloroform extract was 604.50 µg/ml for the 48th hour in HT-29 cell lines, and the 48th hour in A549 cell lines. determined. In HT-29 cell lines, the IC50 effective dose of water extract was determined as 2239.00 µg/ml, the IC50 effective dose of methanol extract was 69.81 µg/ml, and the IC50 effective dose of chloroform extract was 69.46 µg/ml for the 48th hour (Figure 2.).

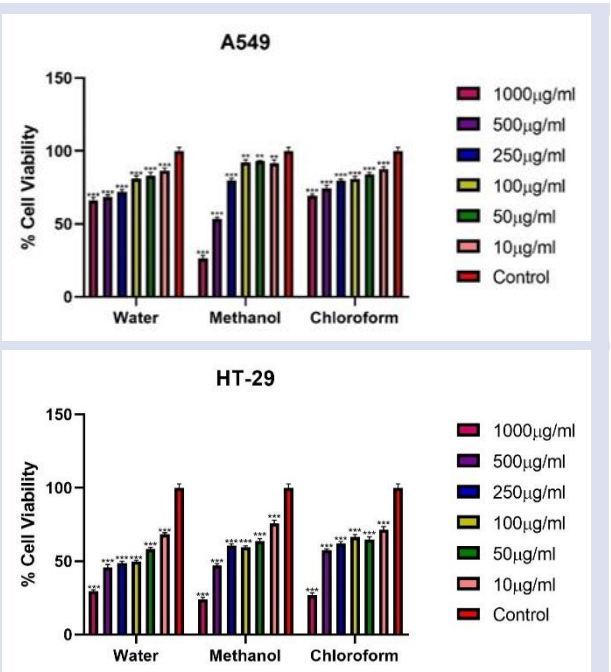
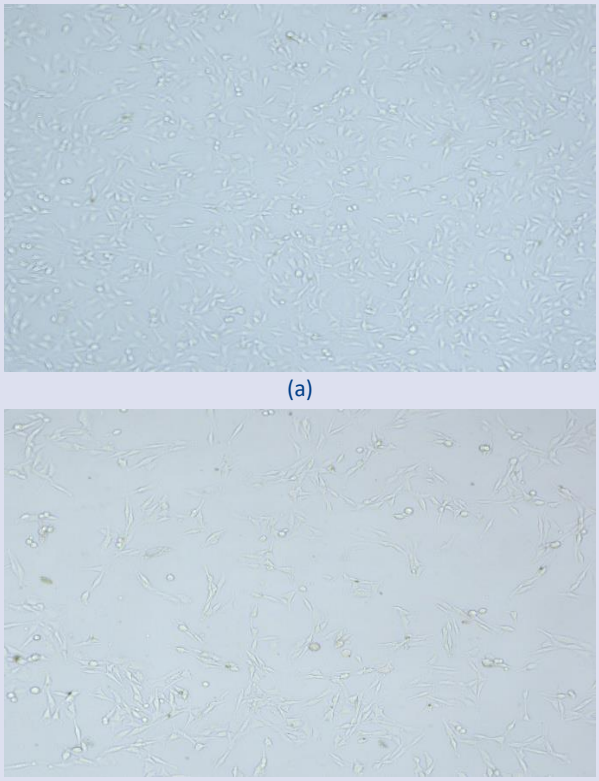


Figure 3. Viability percentages of A549 and HT-29 after 72 h incubation (***P < 0.001, **P < 0.01, *P < 0.05).

In A549 cell lines, the IC50 effective dose of the water extract was determined as 8169.00 µg/ml, the IC50 effective dose of the methanol extract was 544.10 µg/ml, and the IC50 effective dose of the chloroform extract was 29258.97 µg/ml for the 72nd hour. In HT-29 cell lines, the IC50 effective dose of water extract was determined as 139.00 µg/ml, the IC50 effective dose of methanol extract was 244.40 µg/ml, and the IC50 effective dose of chloroform extract was 399.00 µg/ml for the 72nd hour (Figure 3.).

Microscope images

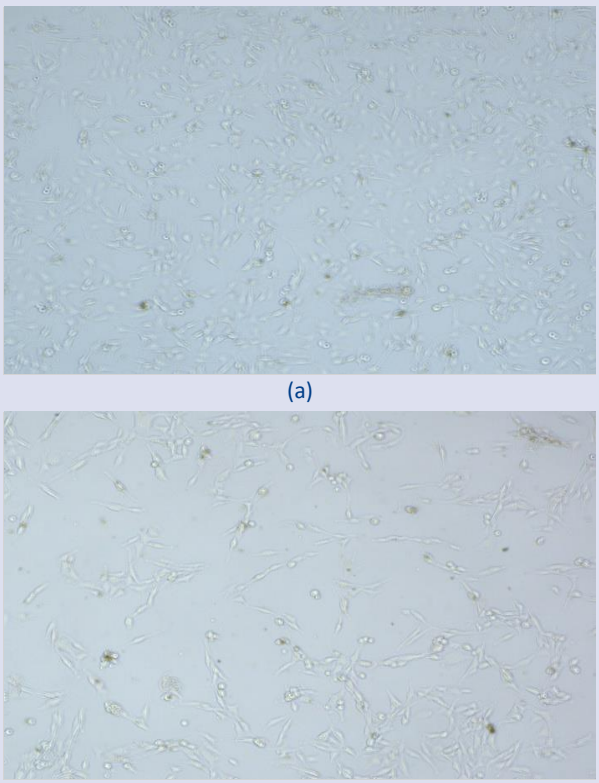
Microscope images of A549 and HT-29 cell lines exposed to 50 µg/ml concentration of water, methanol and chloroform extract of Taraxacum officinal plant at 24h were presented (Figures 4-7.).



(a)

(b)

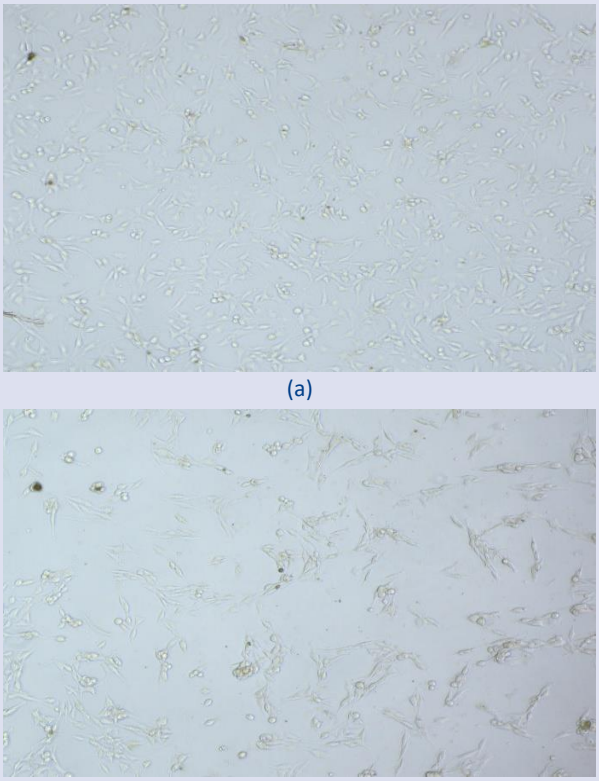
Figure 4. Microscope images of (a) A549 and (b) HT-29 cell lines exposed to water extract of Taraxacum officinal plant for 24h.



(a)

(b)

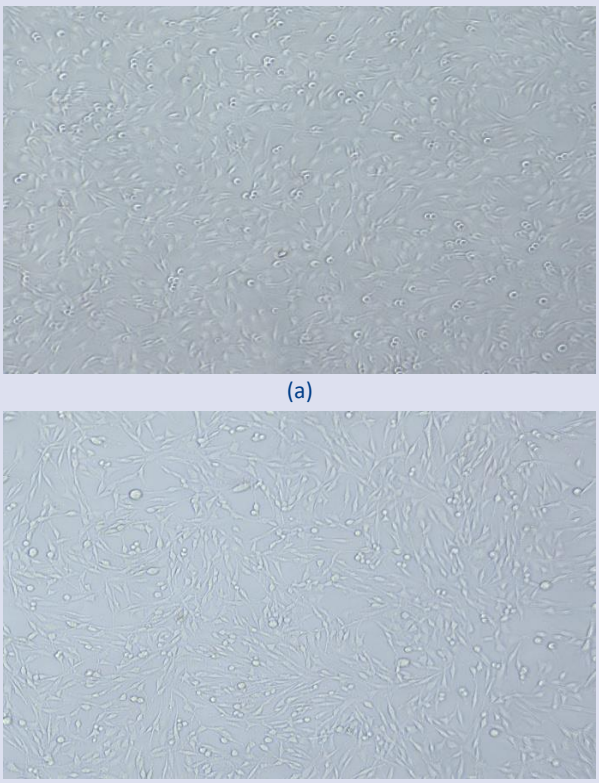
Figure 5. Microscope images of (a) A549 and (b) HT-29 cell lines exposed to methanol extract of Taraxacum officinal plant for 24h.



(a)

(b)

Figure 6. Microscope images of (a) A549 and (b) HT-29 cell lines exposed to chloroform extract of Taraxacum officinal plant for 24h.



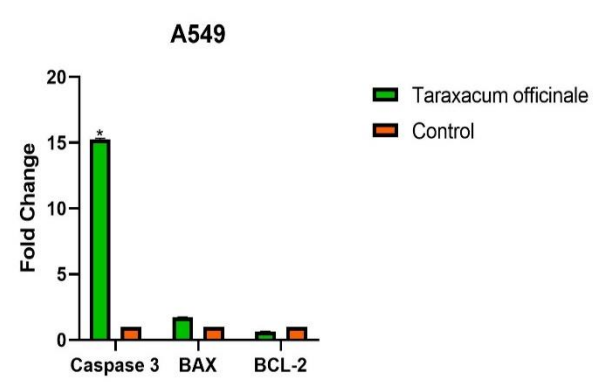
(a)

(b)

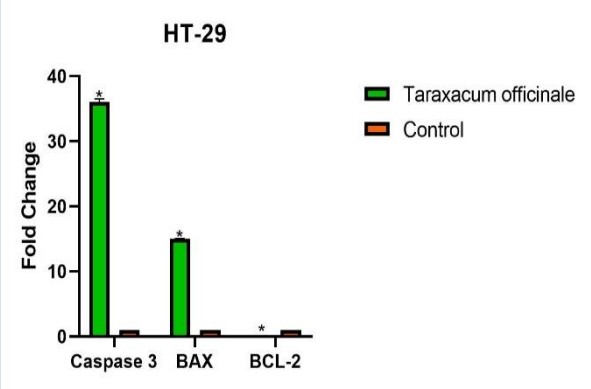
Figure 7. Microscope images of control group (a) A549 and (b) HT-29 cell lines at 24h.

BAX, BCL-2 and Caspase-3 Expression Levels

Caspase 3, BAX and BCL-2 expression levels were determined in cDNA samples obtained from A549 and HT-29 cell lines and control group cell lines, to which the effective dose of chloroform extract of Taraxacum officinal plant was applied for 24 hours (Figure 8.).



(a)



(b)

Figure 8. Caspase 3, BAX and BCL-2 expression profiles in A549 and HT-29 cell lines to which effective dose of chloroform extract of Taraxacum officinal plant was applied.

It was determined that Caspase 3 increased significantly by 15.24 times in A549 cell lines exposed to the effective dose of chloroform extract of the Taraxacum officinal plant at the 24th hour ($p<0.05$). In HT-29 cell lines exposed to the effective dose of chloroform extract of the Taraxacum officinal plant at the 24th hour, BAX increased significantly by 15.03 times, Caspase 3 by 36.02 times, and BCL-2 increased significantly by 10.87 times. It was determined that it decreased ($p<0.05$).

Total Antioxidant Status

TAS levels were determined in four different doses of water, methanol and chloroform extract of Taraxacum officinal plant concentrations between 10µg/ml and 1000µg/ml (Table 1.).

It was determined that the TAS levels of water, methanol and chloroform extracts of the Taraxacum officinal plant increased with increasing concentration, and the highest TAS level was at 1000µg/ml concentrations.

Table 1. TAS levels of water, methanol and chloroform extract of Taraxacum officinal

	Water	Methanol	Chloroform
	TAS (mmol Trolox Equiv. /L)	TAS (mmol Trolox Equiv. /L)	TAS (mmol Trolox Equiv. /L)
10 µg/ml	0,219±0,012	0,491±0,023	0,359±0,019
100 µg/ml	0,256±0,011	0,609±0,026	0,541±0,027
500 µg/ml	0,689±0,031	0,762±0,035	0,677±0,025
1000 µg/ml	1,270±0,037	1,111±0,045	0,889±0,041

The TAS level of the water extract of the Taraxacum officinal plant at a concentration of 1000µg/ml was 1,270 mmol Trolox Equiv. /L, TAS level at 1000µg/ml concentration of methanol extract is 1.111 mmol Trolox Equiv. /L, TAS level at 1000µg/ml concentration of chloroform extract is 0.889 mmol Trolox Equiv /L.

Discussion

There are many studies investigating the effects of Taraxacum officinal on cancer. If we touch upon these studies, it has been shown that Taraxacum officinal extracts strongly inhibit breast cancer stem cell proliferation [21]. It has been determined that ethanol extracts of Taraxacum officinal have a proliferation reducing effect on MCF-7 breast cancer cell lines and WRL-68 liver cancer cell lines [22]. It has also been reported that ethanol extract of Taraxacum officinal flowers has an anti-cancer effect by inducing apoptosis in SK-OV-3 ovarian cancer cell lines [23]. However, in a study examining the anti-cancer activity of DMSO (dimethyl sulfoxide) and ethanol extracts of Taraxacum officinal leaves on glioblastoma, an aggressive type of cancer, alone and in combination with chicoric, chlorogenic, and caftaric acids, it was reported that DMSO extracts showed the best anti-tumor activity and that the anti-cancer activity of the plant was affected by the type of extract and the extract concentration [24]. In another study on glioblastoma regarding Taraxacum officinal, it was determined that DMSO, ethanol and water extracts of Taraxacum officinal leaves and roots all suppressed cell viability in U-138 cancer cell lines. It was reported that ethanol extracts inhibited proliferation at the highest level compared to other extracts [25]. In cancer cell culture studies using the nanomaterial developed by green synthesis method and consisting of Taraxacum officinal leaf extracts and ZnO, it was reported that this nanomaterial had cytotoxic effects on cancer cells. Therefore, it was emphasized that this nanomaterial could be used for cancer treatment with an environmentally friendly approach [26]. In a different study conducted on mouse fibrosarcoma and hepatoma cancer cell lines, it was determined that methanol extracts of Taraxacum officinal had dose-dependent anticancer effects on fibrosarcoma cell lines. In addition, caffeic acid, chlorogenic acid, and ursolic acid isolated from Taraxacum officinal methanol extract were shown to significantly suppress cell proliferation in these two cancer cell lines

[27]. In a study investigating the antioxidant and anti-cancer effects of the essential oil obtained from Taraxacum officinal in vivo and in vitro, it was shown that the essential oil obtained from the plant mediated an increase in antioxidant enzyme levels against oxidative stress induced by paracetamol in mice and protected the liver and kidneys of mice against oxidative damage. In addition, it was determined that Taraxacum officinal essential oil had proliferation inhibitory effects in HeLa cancer cells in relation to the dose used [28]. It was found that hydroalcoholic extracts of Taraxacum officinal had anti-tumor effects on triple-negative breast cancer cells 4T1 and increased apoptosis (by increasing expressions of p53, BAX, Beclin-1 and Atg-7 and BAX/BCL-2 ratio, and also by suppressing BCL-2 expression) and thus decreased proliferation in a dose-dependent manner [13]. It was shown that water and ethanol extracts of Taraxacum officinal prevented cell migration in hepatocyte carcinoma Huh7 cells, which frequently metastasize, and thus had metastasis-inhibiting properties in addition to its anti-cancer properties [29]. In another study, the anti-cancer activity of water, methanol, ethanol and chloroform extracts of the plant was investigated in prostate cancer cell lines PC-3, colorectal cancer cell lines Caco-2, breast cancer cell lines MCF-7 and chronic myeloid leukemia cell lines K562. It was determined that the chloroform extract, which was determined as the most active form of Taraxacum officinal, selectively showed an anti-proliferation effect in prostate and chronic myeloid leukemia cell lines [30]. Finally, it was shown that ethanol extracts of Taraxacum officinal plant roots applied to cervical cancer cell lines caused the induction of apoptosis in cancer cells in a manner closely related to the dose, thus inhibiting proliferation [31]. According to the data of our study, we can say that water, methanol and chloroform extracts of Taraxacum officinal plant have anti-cancer effects in A549 and HT-29 cancer cell lines. If we look at the results of studies in the literature emphasizing the anti-cancer properties of the Taraxacum officinal plant, they seem to be closely related to the results we obtained.

We determined that the chloroform extract of Taraxacum officinal activated cell apoptosis by inducing Caspase 3 expression in A549 cell lines. We found that it induced apoptosis by mediating an increase in Caspase-3 and BAX expression and a decrease in BCL-2 expression in HT-29 cell lines. In a study related to this subject, it was determined that cells were directed to apoptosis by increasing Bax and p53 expression and decreasing Bcl-2 expression in breast cancer 4T1 cells exposed to hydroalcoholic extract of Taraxacum officinal [13]. In a different study, it was found that Taraxacum officinal also had an apoptosis-inducing effect in leukemia cell lines [32]. Based on the results of our study, we can say that the inducing effects of Taraxacum officinal on apoptosis in A549 and HT-29 cell lines are possible through the regulation of different apoptosis-related genes.

In the study investigating the antioxidant capacity in water and ethanol-water extracts obtained from the leaves of Taraxacum officinal plant, it was determined that the antioxidant capacity was quite high. In addition, it was determined that the extracts had a rich polyphenol content. In this respect, it was reported that Taraxacum officinal plant may be effective in the treatment of liver and gall diseases [33]. It was determined that the antioxidant capacity was high in all water-ethanol extracts obtained from the roots, stems, leaves and flowers of Taraxacum officinal by different methods. However, it was determined that the extraction method showing the best antioxidant activity was the Soxlet method [34]. It was shown that the methanol extract was the extract type with the highest phenolic content among the methanol, acetone and n-hexane extracts of Taraxacum officinal. It was reported that the highest antioxidant activity was again found in the methanol extract [35]. In the study where methanol and ethyl acetate extracts of Taraxacum officinal leaves were evaluated in terms of phytochemicals, antioxidant capacity and vitamins, it was reported that the extracts contained high concentrations of phenolic content, flavonoids, alkaloids, vitamins A and C. When methanol and ethyl acetate extracts were compared in terms of antioxidant capacity, it was determined that the highest antioxidant activity was in the methanol extracts of the plant [36]. In a different study where the antioxidant capacity of the fruit extracts of the plant was investigated, it was revealed that the antioxidant content of the fruit extracts of Taraxacum officinal was also high [37]. Similar to these studies in the literature, we determined that the antioxidant status of water, methanol and chloroform extracts of Taraxacum officinal plant was high, that the antioxidant capacity increased dose-dependently with increasing extract concentrations and that the methanol extract of the plant had the highest antioxidant activity.

Conclusions

In conclusion, in this study where we examined the anti-cancer effects of Taraxacum officinal plant in lung and colon cancer, we determined that the extracts of this plant prevented proliferation in A549 lung and HT-29 colon cancer cells. We showed that Taraxacum officinal achieves this by regulating gene expressions in the apoptosis pathway in a way that induces apoptosis in cancer cells. Using these data obtained from our study, we can say that Taraxacum officinal plant may have therapeutic properties in lung and colon cancer. However, in order to express this prediction more strongly, more advanced and in-vivo studies related to Taraxacum officinal and cancer are needed.

Conflicts of interest

There are no conflicts of interest in this work.

Acknowledgement

There is no acknowledgement.

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