European Review for Medical and Pharmacological Sciences 2022; 26: 1462-1474

In vitro investigation of cytotoxic, antioxidant, apoptosis-inducing, and wound healing properties of endemic *Centaurea fenzlii* Reichardt extracts

E.N. ŞIMŞEK SEZER

Department of Biology, Faculty of Science, Selçuk University, Konya, Turkey

Abstract. – OBJECTIVE: Cancer is a formidable problem for global health, and the increasing burden necessitates the search for new and alternative treatments and/or approaches. For this reason, any approach to cancer treatment is extremely valuable. Recently, there has been increasing interest in the use of plant-derived compounds in the treatment of cancer. Many studies have shown that many of the best-selling anti-cancer drugs are of plant origin. Based on this, the cytotoxic potential of two extracts [*C. fenzlii* leaf extract (CFL) and *C. fenzlii* capitula extract (CFC)] from *Centaurea fenzlii*, a plant species endemic to Turkey, were tested in two different cancer cell lines (DLD1 and ARH77) and a healthy cell line (HUVEC).

MATERIALS AND METHODS: Cytotoxic effects of the extracts were determined by MTT assay, changes in the mRNA expressions of the apoptotic genes (*apaf*, *bax*, *bcl2, bcl2l11*, *casp3*, *gadd45a* and *hrk*) were determined *via* the qRT-PCR technique, and the caspase3 enzyme activity was determined. In addition, the capacities of the extracts against invasion and migration were also evaluated. The chemical content and phenolic composition were evaluated *via* the gas chromatography-mass spectrometry (GC/MS) and high-performance liquid chromatography (HPLC) techniques.

RESULTS: As a result, the extracts showed a selective cytotoxic effect in all the cancer cell lines examined, especially the DLD1 colorectal cell line. In addition, it has been determined that the extracts inhibit the invasion and migration of cells.

CONCLUSIONS: The results of the study reveal that *C. fenzlii*, which has been found to have acceptable anti-cancer effects, should be investigated with more comprehensive studies.

Key Words:
Battalbaş, Endemic, MTT, Wound-healing, Turkey.

Introduction

Cancer is a global health issue that requires the search for new and innovative approaches. Despite the increasing burden of cancer globally, there is still a lack of sufficient progress in reducing its mortality and morbidity. This issue requires the search for new treatments and approaches[1]. It was estimated that 17 million new cancer cases were diagnosed in 2018, and more than half of these patients died. Moreover, reports[2] have indicated that the incidence of new cases will increase to 27.5 million by 2040. Plants are recognized as important resources for researchers to discover and develop new drugs. The use of plants in cancer treatment has a long history and, thus, plants have often been the primary sources for the production of effective traditional medicines in cancer treatment[3]. A large number of plant species have been reported to be used in cancer treatment since ancient times in various studies. Today, due to the inadequacy in cancer treatments, the tendency to evaluate the therapeutic effects of plants and plant-derived compounds as potential anti-cancer drugs has increased. This trend emerges when natural products are associated with conventional treatments, suggesting that nutraceutical supplements may contribute to anti-cancer treatment. It has been reported[4] that plant-derived compounds, which are mostly characterized by polyphenolic profile, show their effects in anti-cancer treatment as inhibition of cell proliferation, antioxidant and anti-inflammatory. Curcumin is one of the best-known polyphenols and has shown numerous therapeutic benefits against inflammation, oxidative damage, obesity, metabolic syndrome, neurodegenerative diseases, and various cancers. In addition, curcumin has been reported to inhibit the growth of many tumors, inhibit cell growth, block the cell cycle, and induce apoptotic death[5]. Quercetin is a member of the class of polyphenolic flavonoid compounds almost ubiquitous in plants and plant food sources. By a number of its actions on the cells, it is known as a potential anti-cancer agent, including cell cycle regulation and tyrosine kinase inhibition[6-8]. More importantly, quer-

Corresponding Author: Ela Nur Simsek Sezer, Ph.D; e-mail: elasimsek@selcuk.edu.tr

cetin has been shown to increase the therapeutic efficacy of different chemotherapeutic drugs like cisplatin both in cells and in animal models[9]. Its general effect mechanism is related to its ability to alter cell cycle progression, promote apoptosis, inhibit cell proliferation, inhibit the progression of metastases and angiogenesis[10]. Unfortunately, the main limitation in the use of polyphenols for cancer treatments is their low bioavailability due to oxidation and polymerization in biological fluids. Therefore, there is a need for nano-formulations that can enter cells and increase their efficiency. In previous studies[11,12], it was reported that when polyphenols were loaded with hyaluronic acid, they inhibited cytokines that play a role in cell survival in thyroid and brain cancers. As a result, it is understood from these studies that not only should natural compounds be brought together or show a crude synergistic effect, but also biological activation should be provided through an appropriate natural carrier molecule. For this reason, cancer-fighting scientists have recently focused on the use of natural molecules (both as anticarcinogenic agents and bioactive carrier molecules) in combination with chemotherapy to reduce the side-effect profile and positively affect the treatment process.

The genus *Centaurea* L. is a large genus that contains about 500-600 species, distributed all over the world. Many species belonging to the genus are used in folk medicine for the treatment of various disorders[13]. *Centaurea* species are traditionally used to treat various ailments, such as abscesses, stomachache and headache, asthma, hemorrhoids, gastrointestinal symptoms, cardiovascular diseases, and parasitic and microbial infections[14,15]. In addition, many recent ethno-pharmacological studies have reported that *Centaurea* species have antioxidant, antiradical, antibacterial, antimicrobial, anti-inflammatory, cytotoxic, and apoptotic properties[16-20]. *Centaurea fenzlii* is a species endemic to Turkey, and separate extracts from its flowers and leaves were obtained in this study. Herein, it was aimed to 1) determine the chemical contents of these extracts with different analytical approaches and 2) evaluate the cytotoxic and anticarcinogenic effects of extracts obtained from different parts of *C. fenzlii* on DLD1 and ARH77 cells. For this purpose, various approaches, such as 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), quantitative real-time polymerase chain reaction (qRT-PCR), caspase3, and invasion and migration tests were applied. With this in mind, it is hoped that this article will be a source of inspiration for the development of phytotherapeutic drugs, as limited studies on pharmacological properties and potential anti-carcinogenic agents have been conducted on this taxon.

Materials and Methods

Cell Culture and Chemicals

The ARH77 (multiple myeloma), DLD1 (colorectal cancer) and HUVEC (umbilical vein endothelial) cell lines were selected to determine the cytotoxic activities of the extracts. The cell lines were kindly provided by Dr. Ali Uğur Ural and Dr. Ferit Avcu. Cells were incubated in RPMI-1640 basal medium supplemented with 2 mM of L-glutamine 10% FBS and 1% penicillin-streptomycin at 37°C, and 5% CO_2 until reaching the desired confluence.

Plant Material and Preparation of the Extracts

Centaurea fenzlii was collected from Muş-Bulanık, Varto Road, Muş-Korkut, around the turn, field edges, 370712 N, 421030 E, 1460 m, 22.06.2020. The plant sample was collected by Dr. Kuddisi Ertuğrul and Dr. Tuna Uysal and identified by the second collector. The voucher specimen is preserved in the KNYA herbarium under-sample code KE-6115. The general view of the plant and the capitula image are given in Figure 1. The aerial parts of the plant were dried in the dark and at room temperature. After the plant was separated into leaves and capitula, it was pulverized with a mechanical grinder. The powdered plant materials were extracted by ultrasonic extraction. Sonication was carried out with methanol for 60 min using an ultrasonic cleaning bath. The temperature was checked frequently and kept at the desired level. The extract was filtered and evaporated[21]. Prepared extracts were stored at -20°C until further analysis. The extracts were coded as *C. fenzlii* leaf extract (CFL) and *C. fenzlii* capitula extract (CFC), respectively.

High-performance liquid chromatography analyses

For the detection of phenolic compounds in the extract, 100 mg of each extract sample was dissolved in methanol and the final volume of the solution was adjusted to 10 mL and the mixture was filtered through a 0.22-µM sterile filter. High-performance liquid chromatography (HPLC) analysis was per-

formed at the Selçuk University Research and Development Centre. Analyses were performed using a Shimadzu LC-20AD instrument equipped with a UV/VIS (SPD-20A) detector and an INERTSIL ODS-3V (5 µm; 4.6 × 250 mm) column. The method used in the analyses by Wen et al[22], was used with some minor modifications. The constant flow rate of the solvent system is 1.0 mL/min. The mobile phase was 0.05% glacial acetic acid (solvent A) and ACN (solvent B). The gradient was as follows: 0.1-2 min, 8%-10% B; 2-27 min, 10%-30% B; 27-37, 30%-56% B; 37-37.1 min, 56%-8% B; 37.1-45 min, 8% B; and 45-45.1 min, 8% B. The study was carried out at a temperature of 30 °C and a wavelength of 280 nm. It was studied with a 20-µL injection volume. In this study, 11 phenolic substances were screened and their amounts were determined. The used standards comprised phenolic acids (protocatechuic, caffeic, syringic, benzoic, trans-cinnamic, trans-ferulic and trans-p-coumaric) and flavonoids (trans-resveratrol, rutin trihydrate, catechin hydrate, and naringenin). The standards were prepared in HPLC-grade methanol. The total analysis time was exactly 45.10 min. HPLC analyses were performed with at least 3 parallel tests for both the standards and the extracts, and the mean values were taken into consideration.

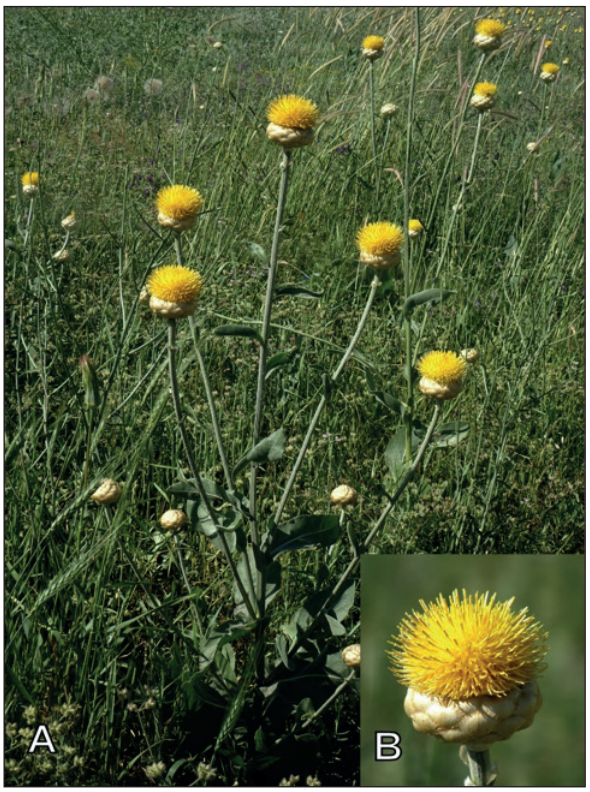

Figure 1. A, General view of the *C. fenzlii*. **B**, capitula part of the plant specimen.

Gas Chromatography-Mass Spectrometry Analyses

The composition of the *C. fenzlii* extracts was determined by gas chromatography-mass spectrometry (GC-MS). All analyses were carried out on a Thermo Fischer Scientific ISQ 7000 Single Quadrupole GC-MS System (Thermo Electron Corporation, Waltham, MA, USA) with a FAME capillary column (30 m × 0.25 mm i.d.; film thickness of 0.25 µm). Helium was used as a carrier gas, at a flow rate of 1.0 mL/min. The oven temperature was programmed to increase from 40 to 240°C at a rate of 5°C/min, and then held isothermally for 12 min; the total run time was 54 min. Identification was based on the comparison of their RI with those previously reported and by matching their mass spectra with those of Wiley 9 Library or literature data. The GC/MS analysis of the extracts was carried out at the ESOGU Research and Development Centre.

Antioxidant Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis was carried out according to a slightly modified version of the method described by Brand-Williams et al[23]. DPPH (Sigma-Aldrich GmbH, Sternheim, Germany) solution in ethanol was solved for 30 min and the absorption of the solution was adjusted to 1.0 ± 0.1 at 490 nm. Then, 200 µL of sample and standard were mixed with 800 µL of DPPH solution and incubated at room temperature and in the dark for 30 min. Absorption was measured at 490 nm. Ascorbic acid was used as a reference. Analyses were made in triplicate repetitions. The scavenging capacity of the extract was expressed as the half-maximum inhibitory concentration (IC_{50}).

Determination of the Total Phenolic Content

The total phenolic content (TPC) of each extract was evaluated according to the previous method[24,25]. Each extract was prepared by dissolving 1 mg/mL. The mixture was incubated in an ultrasonic bath for 5 min to obtain a well-mixed solution. To 300 µL of this solution taken in a tube, 3.16 mL of distilled water, 1 mL of methanol, and 200 µL of Folin reagent were added. Then, after incubation at room temperature, 600 µL of Na_2CO_3 solution was added and the tube was covered with aluminum foil and incubated in a water bath at 40°C for 30 min and measured at

765 nm. The gallic acid standard curve was obtained using the same procedure.

Cytotoxicity Assay

Cytotoxicity was studied by the ability of cells to cleave the MTT tetrazolium salt (Sigma, Chem, St. Louis, MO, USA). Cells were seeded in 96-well plates at 1×10^4 cells/well and incubated at 37°C for 24 h. Prepared were 5 doses (0.0625-1 mg/mL) by serial dilution from the extracts prepared as 1 mg/mL stock solution and applied to cell lines for 48 h. The negative control was performed using the growth medium alone, while vinblastine was used as the positive control. At the end of the incubation period, MTT was added to the cells and allowed to incubate for 4 h. Added to each well was 100 µL of isopropanol to dissolve the formazan crystals formed at the end of the period[26]. The plates were read at 540 nm in an ELISA reader. The effect of the extracts on cell viability was calculated by comparing the absorbance values of the control group (no treatment). Analyses were performed in triplicate. Mean values were considered for cell viability values.

Real-Time PCR

The total RNA of the DLD1 cells was extracted using an Aurum Total RNA Isolation kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions, and the quality and concentration values of the RNA samples were measured using Nanodrop 2000 (Wilmington, DE, USA). Next, 0.5 microgram of total RNA was reverse transcribed into cDNA using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). The mRNA expression levels of the β-actin and apoptotic gene regions were evaluated by real-time PCR. The real-time primers were selected from the human apoptosis primer library HPA-I (Real-Time Primers, LLC, Elkins Park, PA, USA). Gene expression experiments were performed in a 10 µL final volume containing 5 µL of SYBR green master mix (Bio-Rad, Hercules, CA, USA), 1 µL cDNA, 3 µL dH_2O, and 1 µL primer. β-actin was used as the reference gene used as an internal control for normalization. Melting curve analyses were performed and the specificity of the amplicons was confirmed by the presence of a single peak. Data were analyzed using the comparative CT method and the fold change was calculated with $2^{-\Delta\Delta CT}$.

Caspase 3 Assay

The activity of the caspase 3 enzyme was evaluated using the Ab caspase 3 assay kit (Abcam, Cambridge, MA). First, 1×10^6 cells were prepared for each sample and the cells were suspended in 50 µL of tissue lysis buffer and then left on ice for 10 min. The homogenate was then centrifuged, after which the supernatant was transferred to a new tube. Protein concentrations of samples were measured and adjusted 50-200 µg. 2X sample buffer and DEVD-PNA were added to each sample. After 2 h of incubation at 37 °C, the absorbance of the samples was measured at 405 nm using an ELISA reader. The results were compared with the control group and converted into a graph.

Invasion Assay

Invasion experiments carried out *via* CytoSelect Cell Invasion Assay Kit (Cell Biolabs, San Diego, CA). Cell culture inserts placed in 12-well plates were rehydrated with serum-free cell media. The rehydration solution in the insert was then carefully aspirated. Next, 300 µL of cell suspension ($0.5\text{-}1.0 \times 10^6$ cells/mL) and IC_{50} doses of the extracts were added into each insert. After that, the nutrient medium in the insert was carefully aspirated. The ends of 2 to 3 cotton-tipped swabs were wet with water and used to remove non-invasive cells, and the interior side of the inserts was gently swabbed. The inserts were transferred into a clean well that contained cell stain solution and left at room temperature for 10 min. The stained inserts were gently washed several times in a beaker of water. Each insert was transferred to an empty well, the extraction solution was added, and the wells were incubated for 10 min on an orbital shaker. Next, 100 µL of each sample was transferred to a 96-well plate and measured at 560 nm. The invasion capacities of the cells were calculated by comparing them with the control group.

Wound-Healing Assay

Wound healing assay as a model experiment was performed to determine the effect of extracts on DLD1 cells migration. Briefly, the cells were plated into 24-well plates (1×10^5 cells/well) and grown until they were confluent. Then, wounds were generated with a sterile 200-µL pipette tip and washed with 1 × DPBS to remove cell debris, and treated with the IC_{50} doses of each extract. Wound zones were photographed at 0 and 48 h using a Leica DMI LED microscope. The gap closure area was measured by using Image J (National Institute of Health, Bethesda, MD, USA) software.

Table I. Phenolic compositions of the *C. fenzlii* extracts identified by HPLC [ppm (mg/L)] (SD: standard deviation, ND: not detected).

	CFL		CFC	
Phenolic	**Mean**	**SD**	**Mean**	**SD**
Syringic Acid	0.04	0.00	0.017	0.01
Trans-P-Coumaric Acid	0.023	0.01	0.1	0.03
Trans-Ferulic Acid	0.087	0.01	1.44	0.01
Benzoic Acid	0.95	0.34	3.3	0.05
Trans-Resveratrol	0.02	0.00	0.04	0.00
Trans-Cinnamic Acid	ND	-	0.01	0.00
Naringenin	ND	-	0.097	0.04
Protocatechuic Acid	0.32	0.02	1.97	0.12
Catechin Hydrate	**20.92**	0.24	1.90	0.04
Caffeic Acid	0.46	0.37	2.12	0.02
Rutin Trihydrate	0.95	0.02	**20.50**	2.20

Statistical Analysis

Each experiment was repeated in triplicate. Statistical analysis was performed *via* GraphPad Prism 9 for Windows (Graph Pad Software, San Diego, CA, USA). Data were compared using 1-way ANOVA and the post-hoc Tukey test (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Results

Phytochemical Content

Different parts of *C. fenzlii* were extracted with methanol and the phenolic content was determined *via* the HPLC method. It is known that phenolic compounds have various biological activities, such as anti-cancer, anti-inflammatory, antioxidant, and antimicrobial[27,28]. Determining the antioxidant activity and phenolic content in plant materials is the first step towards comprehending potential health benefits. Extracts differ in terms of phenolic content and amount according to the plant part they are prepared from. The phenolic compounds in the content of the two extracts and their amounts are given in the Table I. As seen in Table I, the highest phenolic component in CFL extract is catechin hydrate (20.92 ± 0.24 ppm), during routine trihydrate (20.50 ± 2.20 ppm) in CFC extract. On the other hand, the presence of trans-cinnamic acid and naringenin could not be detected in the content of CFL extract.

In addition to the determination of phenolics by HPLC, GC-MS analysis was performed to determine the phytochemical content of the extracts. The main plant components in the extracts are presented in Table II along with their peak areas. According to the results of the analysis, it was determined that the two extracts were considerably different from each other. Two common components were determined in the contents of the extracts. One of them, amitriptyline, which was found in high proportions in both extracts, is effective in the treatment of irritable bowel syndrome. The positive effect of the extracts on colorectal cancer cell lines may be due to this component. The most abundant metolcarb in the CFL, is a carbamate ester, and the anti-cancer properties of carbamate ester derivatives have been previously reported[29]. On the other hand, the most abundant Bis[2-(3,4-dihydroxy-4-bromophenyl)-1-cyanoethenyl] sulfonate in the CFC was a catechol, and catechols were found to have potent anti-cancer properties against tumor cells in previous reports[30].

Antioxidant Potential of the Extracts

The antioxidant activity of the *C. fenzlii* extracts was evaluated using the DPPH scavenging assay and the IC_{50} values are presented in Table III. According to the DPPH analysis results, the extract with the most radical scavenging effect was CFC (IC_{50}: 0.166 ± 0.01 mg mL^{-1}). The phenolic contents of the extracts (TPC) were determined according to the equation obtained from the gallic acid standard curve graph ($y = 0.0048x + 0.0156$; $R^2 = 0.9871$) and the flavonoid contents of the extracts (TFC) were calculated with the equation obtained from the routine standard curve ($y = 0.0011x + 0.0932$; $R^2 = 0.9733$). The obtained data revealed that the highest phenolic and flavonoid contents were in extracts from the capitula, consistent with the DPPH results.

Cytotoxic Potential of the Extracts

Herein, the obtained extracts were applied for the first time to DLD1 and ARH77 cell lines, and the cytotoxic potential of the *C. fenzlii* extracts was evaluated *via* MTT assay, against human can-

Table II. GC/MS analysis of the *C. fenzlii* extracts (CFL and CFC).

Compounds	% Area	
	CFL	CFC
Metolcarb	**23.54**	-
Amitriptyline	**13.28**	12.38
Silane, trimethyl(phenethylthio)	6.72	-
2-phenyl-2-methyl-1-D1-aziridine	6.43	-
2-(allyldimethylsilyl)pyridine	5.18	-
Hexylresorcinol	8.67	-
2-naphthalenecarboxamide,3-hydroxy-4-[2-(2-methyl-5-nitrophenyl)diazenyl]-N-phenyl-	10.55	-
2-(5-(5-[cyano-(9,9-dimethyl-1,4-dioxa -7-aza- piro[4.4]non-7-en-8-yl)-methylene]-3,3-dimethylpyrrolidin-2-ylidenemet hyl)-3,3-dimethyl-ë1-pyrrolin-5-ylidene methyl-4,4,5-trimethyl-ë1-pyrroline-5-carbonitrile]	11.47	5.83
10,13-dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2-carboxylic acid,4-methyl-11-(1-propenyl)-, methyl ester	-	10.80
Striatal B	-	7.01
3,4,5-trimethoxybenzoic acid	-	**20.55**
Bis[2-(3,4-dihydroxy-4-bromophenyl)-1- cyanoethenyl]sulfonate (catechol)	-	**33.29**
[2-(N-acetylanilino)-1,3-selenazol-4-ylmethyl]triphenylphosphonium iodide	-	3.73
Dihydroobscurinervinediol diacetate(ester)	-	6.42
Total area	100	100

cer cell lines (DLD1 and ARH77) and human vein endothelial cells (HUVEC). The extracts showed a dose-dependent cytotoxic effect on the cell lines. The dose-dependent effect of the extracts on cell viability is given in Figure 2. The IC_{50} values obtained from the graphs are presented in Table IV. The IC_{50} values of the extracts ranged from 53.6 to 541 µg/mL, while the IC_{50} values of VBL were between 9.6 and 13.3 µM. The selectivity index is an important indicator of the therapeutic applicability of the applied extract or phytochemical and gives information about how selectively it can destroy cancer cells compared to normal cells[31,32]. The selectivity index of each extract is given in Table IV. The most selective cytotoxic effect of the extracts was determined on the DLD1 cells.

mRNA Expression Levels of Apoptotic Gene Regions

In addition to determining cell death, it is very important to determine the cause of cell death. For this purpose, mRNA expression levels of some apoptotic gene regions (apaf, bax, bcl2, bcl2l11, casp3, gadd45a, and hrk) after extract application were determined using the RT-PCR technique. Although the *C. fenzlii* extracts (CFL and CFC) have different effects on the anti/proapoptotic gene expressions, these effects are generally in the direction of upregulation of expression in pro-apoptotic gene regions. The graph showing the relative mRNA expression levels of the gene regions compared to the control is given in Figure 3. When the mRNA expression levels were evaluated in terms of the CFL, the mRNA expressions of the pro-apoptotic gene regions apaf, bax, bcl2l11, hrk, gadd45a, and casp3 increased significantly, by 5.38-fold, 4.4-fold, 2.32-fold, 4.2-fold, and 3.09-fold, respectively. On the other hand, the mRNA expression of the anti-apoptotic bcl2 gene was significantly decreased after extract administration. The mRNA expression levels were assessed after CFC administration, and it was observed that the expression levels of the pro-apoptotic

Table III. DPPH radical scavenging capacity, total phenolic and flavonoid content of the *C. fenzlii* extracts (CFL and CFC).

	CFL	CFC
DPPH IC_{50} values (mg mL^{-1})	0.283 ± 0.03	0.166 ± 0.01
TPC (µg mL^{-1} GAE)	33.92 ± 0.2	38.47 ± 0.39
TFC (µg mL^{-1} Rutin)	4.81 ± 0.05	6.18 ± 0.07

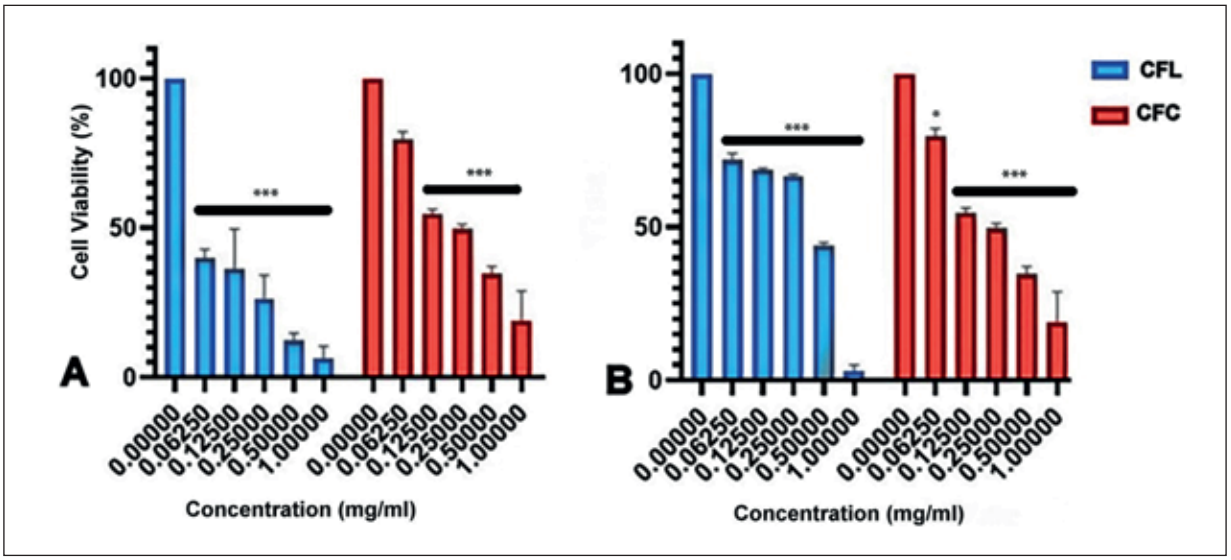

Figure 2. Cytotoxic effects of the *C. fenzlii* extracts determined by MTT assay (A: DLD1; B: ARH77; CFL and CFC).

gene regions, other than casp3, were up-regulated, similar to the CFL. The apaf, bax, bcl2l11, hrk, and gadd45a mRNA expressions increased by 2.2-fold, 5.6-fold, 6.4-fold, 3.5-fold, and 4.3-fold, respectively. On the other hand, the mRNA expression of the casp3 and bcl2 genes did not change significantly after the administration of the extract.

Caspase 3 Activation

Apoptosis is a tightly arranged process by the Bcl2 family and the caspase protein family[33]. Caspases play an important role in mediating a variety of apoptotic signaling pathways. In the caspase cascade causing cell death, caspase 3 is the dominant executioner caspase. In this study, the activity of caspase 3 was evaluated in DLD1 cells treated with *C. fenzlii* extracts for 48 h. As shown in Figure 4, the CFL increased the caspase-3 activity by approximately 1.28-fold when compared to the control, which was consistent with the mRNA expression. These results suggested that CFL induces apoptosis. On the other hand, no significant changes were detected regarding the CFC casp3 activity, such as the mRNA expression.

Inhibition of Invasion and Migration

Cell migration is a very important process in which cells must be able to change in a certain environment and reach their appropriate positions in order to perform their functions[34]. In cancer development and progression, invasion and metastasis occur when tumor cells spread from the primary tumor *via* the circulatory and lymphatic systems, invade basement membranes and endothelial walls, and eventually colonize distant organs[35]. Therefore, the detection of phytochemicals that will inhibit or limit the invasion and migration capacity of cells is very valuable in cancer therapy. For this purpose, the potential of the extracts to inhibit invasion and migration was evaluated in this study.

According to the results, both the CFL and CFC reduced the invasion and migration capacity of the DLD1 cells. When the two extracts were compared, the inhibition rates were close to each

Table IV. IC_{50} and selectivity index values of the *C. fenzlii* extracts (CFL and CFC).

	IC_{50} (mg/mL)			Selectivity Index	
Extracts	DLD1 cells	ARH77 cells	HUVEC cells	DLD1 cells	ARH77 cells
CFL	0.0536 ± 0.002	0.434 ± 0.0042	0.541 ± 0.0025	10.1	1.24
CFC	0.25 ± 0.003	0.325 ± 0.0076	0.336 ± 0.0039	1.34	1.03

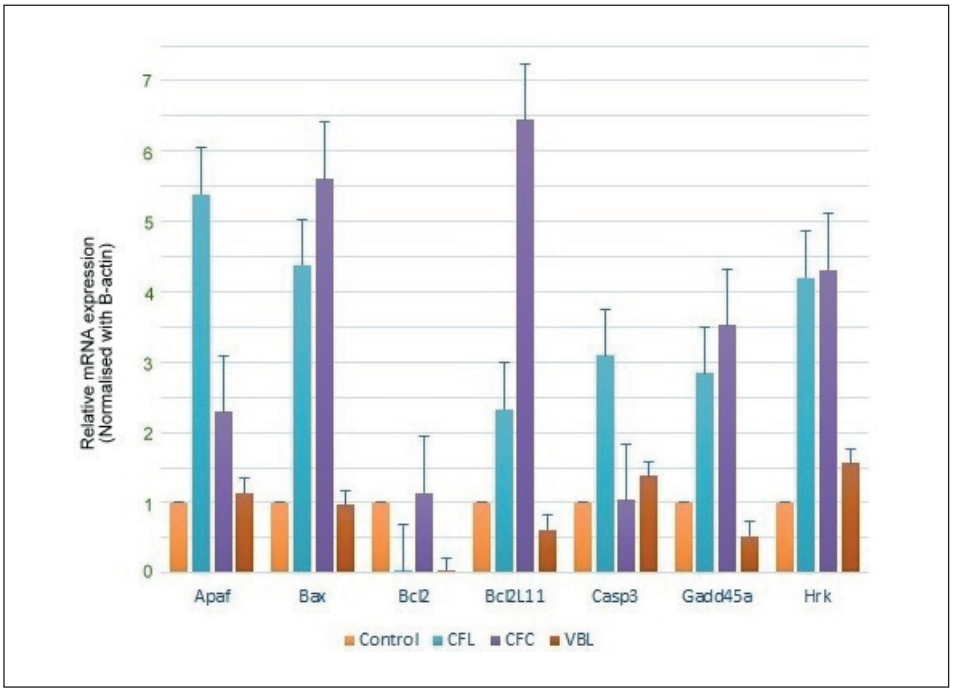

Figure 3. Changes in expression levels of apoptotic pathway-related genes in DLD1 cell line after treatment with the *C. fenzlii* extracts (NT: no treatment (control), CFL and CFC, VBL: vinblastine).

other, but that of the CFL was more effective than that of the CFC. The CFL and CFC strongly reduced the invasion capacity of the DLD1 by 58% and 47% when compared to the control, respectively (Figure 5).

Discussion

In this study, the cytotoxic and apoptosis-inducing effects of extracts obtained from different parts of the *C. fenzlii* plant on two different cell lines, as well as the inhibitory effects of invasion and migration potentials, were revealed. Firstly, the chemical content of the plant extracts was determined and the phenolic compounds detected in this study were consistent with previous reports about *Centaurea* contents[36-38]. There are many reports[39-41] on the cytotoxic/antiproliferative effects of the phenolic compounds observed in this study on different cancer cells. Catechin hydrate, which is the most found phenolic compound in the CFL extract, is a powerful antioxidant and has anti-cancer potential. Previous studies[42,43] have reported that catechin exerts antiproliferative effects on SiHa and MCF-7 cell lines. Rutin is a phenolic found in many plants and has been previously reported to show cytotoxic effects *in vitro* on a variety of cancer cell lines, including human colon cancer[44-46]. Therefore, it can be thought that the cytotoxic effect would be caused by the extracts is due to the phenolic content. From the present literature, it is known that there is limited information about the phenolic content of *C. fenzlii* in previous studies. In a study conducted by Yırtıcı et al[47], the presence of cirsiliol, isorhamnetin, hispidulin, and cirsimaritin in the content was determined by LC-APCI-MS/MS analysis and it was reported that hispidulin was the main component of ethyl acetate fraction of extracts. At the same time, it has been revealed by GC-MS analyzes that many components effective in fighting cancer, such as metolcarb and catechol, are included in the extracts. Therefore, this study can be considered as an important step towards the further investigation of the singular or synergistic but more specific, broader cytotoxic and anticarcinogenic activities of these components[29,30]. On the other hand, it is very important and necessary to eliminate the main limitation of the use of polyphenols in clinical cancer treatments and to increase their bioavailability. Against these clinical limitations, it would be beneficial to develop nano-carriers to

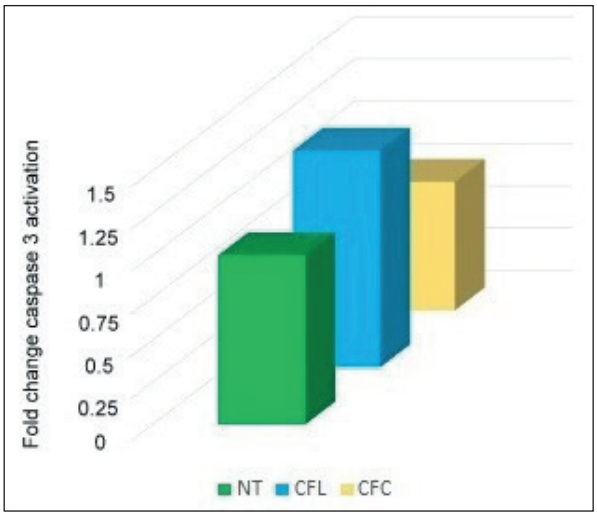

Figure 4. Caspase-3 enzyme levels of the extract-treated DLD1 cell lines (NT: no treatment (control), CFL and CFC).

increase the efficacy of nutraceuticals, protect them from the oxidative and enzymatic environment, and at the same time achieve a more specific and controlled release in cancer cells. Therefore, it is very important for clinicians to develop nano-formulations that facilitate the entry of polyphenols and other nutraceuticals into cells and increase their effectiveness[11,12].

Considering the antioxidant capacities of the extracts, it was found that the total amount of phenolic components of the capitula extracts was higher and accordingly the antioxidant capacity. In a previous study by Yırtıcı[48], the DPPH radical scavenging activity of *C. fenzlii* methanolic extract prepared using the maceration technique was reported as 0.661 mmol TE/g, the phenolic content as 16.72 mg GAE/g and the flavonoid content as 173.16 mg KAE/g. The reported values differed from those in the current study, but this can be explained by the fact that the various bioactive properties (antioxidant, antiproliferative, etc.) and the chemical composition of the plants differ significantly depending on the extraction method, extraction solvent, and varying habitat conditions[49,50].

According to the literature review, this study is the first report on the cytotoxicity of *C. fenzlii* extracts on the DLD1 and ARH77 cell lines. There have been many studies investigating the cytotoxic effects of the extracts of *Centaurea* species. In the study conducted by Yırtıcı et al[47], the dichloromethane ethyl acetate fraction of *C. fenzlii* extract was applied to MCF-7 cells and

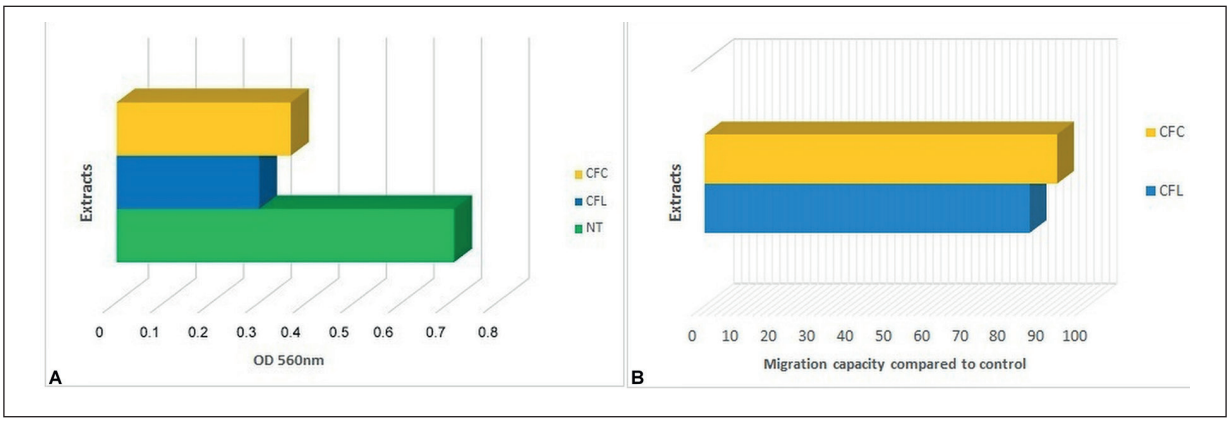

Figure 5. Effects of the *C. fenzlii* extracts on the inhibition of invasion and migration on DLD1 cells, **A**: Invasion capacity of the extracts; **B**: Migration capacity of the extracts (NT: no treatment (control), CFL and CFC.

the IC_{50} dose was reported as 33.121 µg/mL. Shoeb et al[51] reported that methanol extracts of *C. gigantea* had a strong cytotoxic effect on the CaCo-2 colon cancer cell line (IC_{50}: 43.2 µM). Köse et al[15] investigated the biological effects of *C. baseri* from Turkey and reported that the extract showed strong selective cytotoxicity in A549, PANC-1, MCF-7, and C6 glioma cells. Escher et al[52] evaluated the cytotoxic effects of *C. cyanus* petal extracts on Caco-2, A549, and HepG2 cell lines, and reported that the extract showed high IC_{50} (>900 µg/mL) values for all of the cell lines, which indicated a low cytotoxicity and antiproliferative effect. In another study, it was reported that *C. solstitialis* extract caused the inhibition of the percent viability of PC-3 and MCF-7 cells (IC_{50} values: 91.47 µg mL^{-1} and 58.53 µg mL^{-1}, respectively)[53]. Esmaeili et al[54] investigated the cytotoxic effects of methanolic extracts obtained from *C. albonitens* on colon and breast cancer and reported that the methanolic extract did not have a cytotoxic effect on the HT-29 cell line, but had a cytotoxic effect on the MCF-7 cell line (IC_{50}: 69.6 ± 5.7 µg/mL). Taken together, these studies revealed that cytotoxic activity varies depending on the *Centaurea* species chosen, the extraction solvent, and the cell lines used for cytotoxicity assessment. However, it is clear that all *Centaurea* extracts have cytotoxic effects at various levels.

Besides cell death, the way it occurs is also very important. A popular goal of many strategies used in cancer treatment is to induce cells to die by apoptotic pathways. *C. fenzlii* extracts have varying levels of effects on apoptotic gene expressions, these effects are generally positive and are up-regulated in pro-apoptotic gene regions. This is an indication that the extracts stimulate the apoptotic pathway in cancer cells. The findings were in agreement with the findings of a previous study, which reported that the ethyl acetate fraction of *C. fenzlii* showed apoptotic activity against MCF-7 cells, as demonstrated by fluorescent double staining and flow cytometry[47]. In a previous study with a different *Centaurea* species, *C. brugeriana*, the extracts were reported to dose-dependently increase the expression of bax and casp3 and decrease the expression of bcl2 in the MCF7 cell line[55]. Kayacan et al[56] reported that *C. nerimaniae* methanol extract induced apoptotic cell death *via* caspase-3 in a human cervical carcinoma cells HeLa and breast cell line MDA-MB-23. It is a well-known process that changes in the expression of pro/anti-apoptotic genes lead to caspase-3 activation. Although the apoptotic effects of *C. fenzlii* have been demonstrated by different techniques, cell death mediated by dysregulation of apoptosis-related genes was confirmed by RT-PCR in this study.

Caspases are important mediators of apoptosis, and caspase-3 in particular is a frequently activated death protease that catalyzes the specific cleavage of many important cellular proteins[57]. Studies on *Centaurea* extracts and caspase 3 activation are quite limited, but studies generally indicate caspase-dependent apoptosis. In a previous study[58], it was reported that the ethanolic extract of flower parts of *C. solstitialis* caused an increase in caspase-3 activity in both A549 and HeLa cell lines by 1.5-fold and 1.65-fold, respectively. In a study by Kayacan et al[56], it was reported that the extracts obtained from the *C. nerimanie* species caused apoptosis with caspase 3 activation in HeLa and MDA-MB-231 cell lines. In a study by Bahmani et al[59], methanolic extract of another *Centaurea* species, *Centaurea albonitens*, was reported to induce caspase 3-dependent apoptosis in KMM-1, REH, NALM-6, and NB4 cell lines. Thus, this study is in line with previous studies and revealed that CFL induces caspase-dependent apoptosis.

Cancer cell invasion and migration allow the tumor to spread to surrounding tissues and form metastases, which is an important sign of poor prognosis[60]. Therefore, the detection of phytochemicals that will inhibit or limit the invasion and migration capacity of cells is very valuable in cancer therapy. According to the results of this study, it was determined that both *C. fenzlii* extracts reduced the invasion and migration capacity of DLD1 cells. As far as is known from the literature review, there are no reports showing the effects of extracts directly from *Centaurea* species on invasion and migration on cancer cells. However, Aguerin B, a sesquiterpene lactone obtained from *C. behen*, has been reported[61] to have an anti-metastatic effect on MDA-MB-231 breast cancer cells. In summary, in addition to its cytotoxic effect, the inhibition of these two basic mechanisms affecting the metastasis of cells as a result of the application of the extract is very important data and once again reveals the potential of *C. fenzlii*.

Conclusions

Within this study, the cytotoxic and apoptosis-inducing effects of the extracts obtained from *C. fenzlii* and their inhibitory effects on cell inva-

sion and migration were revealed. Although there is another study demonstrating the cytotoxic and apoptotic effects of *C. fenzlii* on MCF7, there are no reports on DLD1 and ARH77. This study was the first to reveal the detailed chemical composition of the plant, its antioxidant, cytotoxic, and apoptosis-inducing effects, as well as its invasion prevention/migration capacity. The data obtained showed that *C. fenzlii* extracts have promising potential in cancer treatment in terms of both content and effect. As a general comment, CFL is more effective against the DLD1 cell line, while CFC is more effective against ARH77. Considering the selectivity index, it is seen that both extracts have a selective effect on the DLD1 cell line, but show lower selectivity on ARH77. Therefore, due to its selectivity, CFL may be the primary resource for developing phytotherapeutic targets for colorectal cancer. Further studies are needed to elucidate the cellular mechanisms underlying this cytotoxic effect and to reveal the full interaction of apoptotic pathways.

Conflict of Interest

The author declares that there is no conflict of interest.

References

1) Bhanot A, Sharma R, Noolvi MN. Natural sources as potential anti-cancer agents: A review. Int J Phytomedicine 2011; 3: 9.
2) Ashraf MA. Phytochemicals as potential anti-cancer drugs: time to ponder nature's bounty. BioMed Res Int 2020; 2020: 1-7.
3) Sewell RD, Rafieian-Kopaei M. The history and ups and downs of herbal medicines usage. J HerbMed Pharmacol 2014; 3: 1-3.
4) Maiuolo J, Gliozzi M, Carresi C, Musolino V, Oppedisano F, Scarano F, Nucera S, Scicchitano M, Bosco F, Macri R, Ruga S, Cardamone A, Coppoletta A, Mollace A, Cognetti F, Mollace V. Nutraceuticals and cancer: Potential for natural polyphenols. Nutrients 2021; 13: 3834.
5) Liyanage PY, Hettiarachchi SD, Zhou Y, Ouhtit A, Seven ES, Oztan CY, Celik E, Leblanc RM. Nanoparticle-mediated targeted drug delivery for breast cancer treatment. Biochim Biophys Acta Rev Cancer 2019: 1871: 419-433.
6) Indap MA, Radhika S, Motiwale L, Rao KVK. uercetin: Antitumor activity and pharmacological manipulations for increased therapeutic gains. Indian J Pharm Sci 2006; 68: 465-469.
7) Yang JH, Hsia TC, Kuo HM, Chao PD, Chou CC, Wei YH, Chung JG. Inhibition of lung cancer cell growth by quercetin glucuronides via g (2)/m arrest and induction of apoptosis. Drug Metab Dispos 2006; 34: 296-304.
8) Kubo I, Nitoda T, Nihei KI. Effects of quercetin on mushroom tyrosinase and b16-f10 melanoma cells. Molecules 2007; 12: 1045-1056.
9) Daker M, Ahmad M, Khoo ASB. Quercetin-induced inhibition and synergistic activity with cisplatin—A chemotherapeutic strategy for nasopharyngeal carcinoma cells. Cancer Cell Int 2012; 12: 34.
10) Potoĭcnjak I, Škoda M, Pernjak-Pugel E, Peršić MP, Domitrović R. Oral administration of oleuropein attenuates cisplatin induced acute renal injury in mice through inhibition of ERK signaling. Mol Nutr Food Res 2016; 60: 530-541.
11) Quagliariello V, Armenia E, Aurilio C, Rosso F, Clemente O, de Sena G, Barbarisi M, Barbarisi, A. New treatment of medullary and papillary human thyroid cancer: biological effects of hyaluronic acid hydrogel loaded with quercetin alone or in combination to an inhibitor of aurora kinase. J Cell Physiol 2016; 231:1784-1795.
12) Barbarisi M, Iaffaioli RV, Armenia E, Schiavo L, de Sena G, Tafuto S, Barbarisi A, Quagliariello V. Novel nanohydrogel of hyaluronic acid loaded with quercetin alone and in combination with temozolomide as new therapeutic tool, CD44 targeted based, of glioblastoma multiforme. J Cell Physiol 2018; 233: 6550-6564.
13) Abad MJ, Bedoya LM, Bermejo P. Fighting Multidrug Resistance with Herbal Extracts, Essential Oils and Their Components: Chapter 14. Essential Oils from the Asteraceae Family Active against Multidrug-Resistant Bacteria; Elsevier Inc. Chapters: 2013.
14) Karamenderes C, Khan S, Tekwani BL, Jacob MR, Khan IA. Antiprotozoal and antimicrobial activities of Centaurea species Growing in Turkey. Pharm Biol 2006; 44: 534-539.
15) Köse YB, İşcan G, Göger F, Akalın G, Demirci B, Başer KHB. Chemical composition and biological activity of *Centaurea baseri*: New species from Turkey. Chem Biodivers 2016; 13: 1369-1379.
16) Ostad SN, Rajabi A, Khademi R, Farjadmand F, Eftekhari M, Hadjiakhoondi A, Khanavi M. Cytotoxic potential of *Centaurea bruguierana* ssp. *belangerana*: The MTT assay. Acta Medica Iranica 2016; 583-589.
17) Radan M, Carev I, Tešević V, Politeo O, Čulić VČ. Qualitative HPLC-DAD/ESI-TOF-MS Analysis, Cytotoxic, and Apoptotic Effects of Croatian Endemic *Centaurea ragusina* L. Aqueous Extracts. Chem Biodivers 2017; 14: e1700099.
18) Bruno M, Modica A, Catinella G, Canlı C, Arasoglu T, Çelik S. Chemical composition of the essential oils of *Centaurea tomentella* Hand.-Mazz. and *C. haussknechtii* Boiss. (Asteraceae) collected wild in Turkey and their activity on microorganisms affecting historical art craft. Nat Prod Res 2019; 33: 1092-1100.
19) Guvensen NC, Keskin D, Gunes H, Oktay MK, Yıldırım H. Antimicrobial property and antiprolif-

erative activity of *Centaurea babylonica* (L.) L. on human carcinomas and cervical cancer cell lines. Ann Agric Environ Med 2019; 26.

20) Hadjira S, Mansour A, Berkel C, Seghiri R, Menad A, Benayache F, Benayache S, Cacan E, Ameddah S. Antioxidant, anti-inflammatory and cytotoxic properties of *Centaurea africana Lamk* var.[Bonnet] M. Antiinflamm Antiallergy Agents Med Chem 2021; 20: 89-100.

21) Stanisavljević I, Stojičević S, Veličković D, Veljković V, Lazić M. Antioxidant and antimicrobial activities of Echinacea (*Echinacea purpurea* L.) extracts obtained by classical and ultrasound extraction. Chinese J Chem Eng 2009; 17: 478-483.

22) Wen D, Li C, Di H, Liao Y, Liu H. A universal HPLC method for the determination of phenolic acids in compound herbal medicines. J Agric Food Chem 2005; 53: 6624-6629.

23) Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 1995; 28: 25-30.

24) Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. Am J Enol Vitic 1977; 28: 49-55.

25) Ahmed D, Khan MM, Saeed R. Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic, and aqueous extracts from *Adiantum caudatum* leaves. Antioxidants 2015; 4: 394-409.

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

27) Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutr Cancer 2009; 62: 1-20.

28) Albuquerque BR, Heleno SA, Oliveira MBP, Barros L, Ferreira IC. Phenolic compounds: current industrial applications, limitations, and future challenges. Food Funct 2021; 12: 14-29.

29) Janganati V, Penthala NR, Madadi NR, Chen Z, Crooks PA. Anti-cancer activity of carbamate derivatives of melampomagnolide B. Bioorganic Med Chem Lett 2014; 24: 3499-3502.

30) Lim WC, Kim H, Kim YJ, Jeon BN, Kang HB, Ko H. Catechol inhibits epidermal growth factor-induced epithelial-to-mesenchymal transition and stem cell-like properties in hepatocellular carcinoma cells. Sci Rep 2020; 10: 1-14.

31) Demir S, Turan I, Aliyazicioglu Y. Selective cytotoxic effect of Rhododendron luteum extract on human colon and liver cancer cells. J Buon 2016; 21: 883-888.

32) Aliyazıcıoğlu Y, Demir S, Yaman SÖ, Şener SÖ, Demir EA, Aliyazıcıoğlu R, Turan İ. Phytochemical analysis of Dorycnium pentaphyllum and its antiproliferative effect on cervix cancer cells. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi 2019; 22: 365-373.

33) Brentnall M, Rodriguez-Menocal L, De Guevara RL, Cepero E, Boise LH. Caspase-9, caspase-3, and caspase-7 have distinct roles during intrinsic apoptosis. BMC Cell Biology 2013; 14: 1-9.

34) Te Boekhorst V, Preziosi L, Friedl P. Plasticity of cell migration *in vivo* and in silico. Annu Rev Cell Dev Biol 2016; 32: 491-526.

35) Fried P, Alexander, S. Cancer invasion and the microenvironment: plasticity and reciprocity. Cell 2011; 147: 992-1009.

36) Azzouzi D, Bioud K, Demirtas I, Gul F, Sarri D, Benayache S, Benayache F, Mekkiou R. Phenolic profile and antioxidant activity of *Centaurea choulettiana* Pomel (Asteraceae) extracts. Comb Chem High Throughput Screen 2016; 19: 841-846.

37) Baykan Erel Ş, Ballar P, Karaalp C. Antioxidant Activities of Phenolic Compounds of *Centaurea ensiformis* PH Davis. Turk J Pharm Sci 2014; 11.

38) Ayad R, Akkal S. Phytochemistry and biological activities of Algerian Centaurea and related genera. Stud Nat Prod Chem 2019; 63: 357-414.

39) Perveen S, Al-Taweel AM. Phenolic Compounds from the Natural Sources and Their Cytotoxicity. Phenolic Compounds Natural Sources, Importance and Applications 2017. Available at: https://www.intechopen.com/chapters/53530.

40) Kasapoğlu KN, Altin G, Farooqi AA, Salehi B, Setzer WN, Sharifi-Rad J. Anti-proliferative, genotoxic, and cytotoxic effects of phytochemicals isolated from Anatolian medicinal plants. Cell Mol Biol 2020; 66: 145-159.

41) Mazumder K, Biswas B, Raja IM, Fukase K. A review of cytotoxic plants of the Indian subcontinent and a broad-spectrum analysis of their bioactive compounds. Molecules 2020; 25: 1904.

42) Alshatwi AA. Catechin hydrate suppresses MCF-7 proliferation through TP53/Caspase-mediated apoptosis. J Exp Clin Cancer Res 2010; 29: 1-9.

43) Al-Hazzani AA, Alshatwi AA. Catechin hydrate inhibits proliferation and mediates apoptosis of SiHa human cervical cancer cells. Food Chem Toxicol 2011; 49: 3281-3286.

44) Menon LG, Kuttan R, Kuttan G. Inhibition of lung metastasis in mice induced by B16F10 melanoma cells by polyphenolic compounds. Cancer Lett 1995; 95: 221-225.

45) Kuntz S, Wenzel U, Daniel H. Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity, and apoptosis in human colon cancer cell lines. European journal of nutrition 1999; 38: 133-142.

46) Guon TE, Chung HS. Hyperoside and rutin of *Nelumbo nucifera* induce mitochondrial apoptosis through a caspase-dependent mechanism in HT-29 human colon cancer cells. Oncol Lett 2016; 11: 2463-2470.

47) Yırtıcı Ü, Göger F, Sarımahmut M, Ergene A. Cytotoxic and apoptotic effects of endemic *Centaurea fenzlii* Reichardt on the MCF-7 breast cancer cell line. Turk J Biol 2017; 41: 370-377.

48) Yırtıcı Ü. *Centaurea fenzlii* Reichardt Özütünün Antioksidan Özellikleri ve Enzim İnhibisyon Etkisinin Belirlenmesi. Bitlis Eren Üniversitesi Fen Bilimleri Dergisi 2019; 8: 66-73.

49) Ganamé HT, Karanga Y, Tapsoba I, Dicato M, Diederich MF, Cerella C, Sawadogo RW. Phytochemical screening and antioxidant and cytotoxic effects of *acacia* macrostachya. Plants 2021; 10: 1353.

50) Le NT, Donadu MG, Ho DV, Doan TQ, Le AT, Ain R, Donatella U, Sanna G, Mauro M, Marianna U. Biological activities of essential oil extracted from leaves of *Atalantia sessiflora* Guillauminin Vietnam. J Infect Dev Ctries 2020; 14: 1054-1064.

51) Shoeb M, Jaspars M, MacManus S.M, Celik S, Nahar L, Kong-Thoo-Lin P, Sarker SD. Anti-colon cancer potential of phenolic compounds from the aerial parts of *Centaurea gigantea* (Asteraceae). J Nat Med 2007; 61: 164-169.

52) Escher GB, Santos JS, Rosso ND, Marques MB, Azevedo L, do Carmo MAV, Daguer H, Molognoni L, do Prado-Silva L, Sant'Ana AS. Chemical study, antioxidant, anti-hypertensive, and cytotoxic/cytoprotective activities of *Centaurea cyanus* L. petals aqueous extract. Food Chem Toxicol 2018; 118: 439-453.

53) Alper M, Özay C, Güneş H, Mammadov R. Assessment of antioxidant and cytotoxic activities and identification of phenolic ompounds of *Centaurea solstitialis* and *Urospermum picroides* from Turkey. Braz Arch Biol 2021; 64.

54) Esmaeili S, Asadi M, Mosaddegh M, Mohammadi Motamed S, Hamzeloo-Moghadam M. Cytotoxic activity of *Centaurea albonitens* Turrill aerial parts in colon and breast cancer cell lines. J Med Plants 2021; 20: 59-67.

55) Nasr FA, Shahat AA, Alqahtani AS, Ahmed MZ, Qamar W, Al-Mishari AA, Almoqbil AN. *Centaurea bruguierana* inhibits cell proliferation, causes cell cycle arrest, and induces apoptosis in human MCF-7 breast carcinoma cells. Mol Biol Rep 2020; 47: 6043-6051.

56) Kayacan S, Sener L, Melikoglu G, Kultur S, Albeniz I, Ozturk M. Induction of apoptosis by *Centaurea nerimaniae* extract in HeLa and MDA-MB-231 cells by a caspase-3 pathway. Biotech Histochem 2018; 93: 311-319.

57) Porter, AG, Jänicke, RU. Emerging roles of caspase-3 in apoptosis. Cell Death Differ 1999; 2: 99-104.

58) Alper M, Güneş H. The anti-cancer and anti-inflammatory effects of *Centaurea solstitialis* extract on human cancer cell lines. Turkish J Pharm Sci 2019; 16: 273.

59) Bahmani F, Esmaeili S, Bashash D, Dehghan-Nayeri N, Mashati P, Gharehbaghian A. *Centaurea albonitens* extract enhances the therapeutic effects of Vincristine in leukemic cells by inducing apoptosis. Biomed Pharma 2018; 99: 598-607.

60) Friedl, P, Wolf, K. Tumour-cell invasion and migration: diversity and escape mechanisms. Nat. Rev. Cancer 2003; 5: 362-374.

61) Amini E, Nabiuni M, Behzad SB, Seyfi D, Eisvand F, Sahebkar A, Shakeri A. Anti-cancer potential of Aguerin B, a sesquiterpene lactone isolated from *Centaurea behen* in metastatic breast cancer cells. Recent Pat Anti-cancer Drug Discov 2020; 15: 165-173.