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BIOMEDICAL SCIENCES

Seseli petraeum M. Bieb. (Apiaceae) Significantly Inhibited Cellular Growth of A549 Lung Cancer Cells through G0/G1 Cell Cycle Arrest

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Abstract: *Seseli* L. is an important genus of the Apiaceae family, with a large number of aromatic species. It is used in traditional medicine extensively, but there is quite limited information on their phytochemicals and biological activities. *Seseli petraeum* M. Bieb. grows in Northern Anatolia, and there are no phytochemical studies on this species. In the present study, we aimed to investigate the effect of the extracts of *S. petraeum* on A549 lung cancer cell proliferation. For this purpose, the antiproliferative effect was determined via MTT assay, and the extracts obtained from the root of *S. petraeum* showed a significant inhibitory effect on cell proliferation. The hexane extract of the root exhibited potent inhibition on A549 cancer cell growth at the 24th hour with 3.432 mg/mL IC_{50} value. The results also showed that the hexane extract had displayed cytotoxic effect through an arrest at the G0/G1 phase of the cell cycle and induced apoptosis as well as DNA damage of A549 cells. Consequently, this study demonstrated the antiproliferative potential of the extracts from *S. petraeum*, especially hexane extract from the roots. Further studies are required to identify the mechanisms underlying these effects.

Key words: A549, Antiproliferative, Apiaceae, Apoptosis, Extract, *Seseli petraeum*.

INTRODUCTION

Aromatic and medicinal plants have attracted attention as a significant source of natural products all over the world (Canter et al. 2005, Singh & Singh 2011, Newman & Cragg 2016). Apiaceae is known as a wealthy family of flowering plants with their aromatic and medicinal properties. It is a cosmopolitan family, and most of the species grow in Asia comprised of 455 genera and over 3700 species throughout the world, mostly in temperate regions (Crowden et al. 1969, Heywood 1979, Davis et al. 1988, Baytop 1999, Pimenov & Leonov 2004, Heywood et al. 2007, Sayed-Ahmad et al. 2017). The *Seseli* L., as old Greek name (Hamlyn 1969), is one of the most prominent genera of Apiaceae with 125 to 140 taxa (Hedge & Lamond 1972, Davis et al. 1988) and 80 of which are distributed in Asia besides Europe, Africa, North America and Australia (Pimenov & Leonov 1993, 2004, Akalın Uruşak & Kızılaslan 2013, Aytaç & Duman 2013) as narrow endemics (Lyskov et al. 2018). On the other hand, *Seseli* is well represented in Flora of Turkey, by 12 taxa (Hedge & Lamond 1972, Davis et al. 1988, Duman 2000, Ozhatay et al. 2009), and new species continue to be discovered (Güner et al. 2011, Güner & Duman 2013, Çetin et al. 2015). Since ancient times *Seseli* is used by Hippocrates and Dioscorides in folk medicine originated from the words “Seseli, seselis, or sesili” (Dioscorides 2002, Stojkovic et al. 2009). *Seseli* species have been widely used in European traditional medicine, exhibiting antibacterial, antifungal,

insect repellent, emmenagogue, anti-flatulence, anti-inflammatory, antinociceptive, anti-tumor, anti-rheumatic activities, and protective effect on human lymphocytes DNA (Ilic et al. 2015). Moreover, the roots of *S. mairei* Wolff. are known as "Zhu Ye Fang Feng" in Chinese folklore and used as a herbal remedy for human inflammation, swelling, rheumatism, pain, and common cold (Hu et al. 1990). In addition, *S. libanotis* is used as a cheese preservative and to provide aroma (Ozturk et al. 2000, Öztürk & Ercişli 2006), and the leaves of *S. libanotis* (Kelemkeşir or kelemenkeşir in Turkish) are consumed as a vegetable especially in the eastern part of Turkey (Baytop 1994). The fruits of *S. tortuosum* are used as an emmenagogue and in digestive diseases (Baytop 1999). Based on traditional uses, many studies concerning biological activities have been performed on *Seseli* species; and found that cytotoxic (Hu et al. 1990, Vuckoviç et al. 2007, 2010, Gonçalves et al. 2012), antimicrobial (Singh et al. 2002, Ozturk & Ercisli 2006, Stojkovic et al. 2009, Matejic et al. 2012, Gonçalves et al. 2012), antioxidant (Stojkovic et al. 2009, Matejic et al. 2012, Stankov-Jovanovic et al. 2015) and anti-inflammatory (Kupeli et al. 2006, Khan et al. 2015, Tosun et al. 2016, Chun et al. 2016) properties.

The high demand and curiosity for natural products, especially for the development of new drugs for the treatment of cancer and other threatening diseases, has led us to examine the cytotoxic activity of plant extracts. The literature search revealed that only a few publications on *S. petraeum* are present (Tosun et al. 2006, Kupeli et al. 2006). *S. petraeum* M. Bieb. is a perennial plant with a specific fibrous collar, called "Taş çaşırı, stone seseli, taş seseli" in Anatolia, grows on rocky slopes in a limited area (Hedge & Lamond 1972). The aim of the present study is to investigate the antiproliferative effects of the extracts from *S. petraeum* obtained by different polarities on A549 cells as lung cancer is one of the leading causes of death in the world. As a part of our ongoing investigations on Turkish *Seseli* species, we undertook the present screening study on *S. petraeum* to evaluate its cytotoxicity potency and apoptosis-inducing effects on A549 cells to determine its probable anticancer properties.

MATERIALS AND METHODS

Plant material

The plant (about 1.5 kg) was collected from the Northern Site of Anatolia (Turkey) called Trabzon-Gümüşhane (Maçka) district (almost 40°28'N and 39°27'E) on 15.07.2017 at an altitude 800 m from the roadside cliffs, in the flowering period. The samples were identified by Prof. Dr. Hayri Duman (Gazi University, Faculty of Science, Department of Biology) and Prof. Dr. Alev Önder (Ankara University, Faculty of Pharmacy, Department of Pharmacognosy). The voucher specimen was kept in AEF (Herbarium of Ankara University Faculty of Pharmacy) under the registration number of AEF 26994.

Extraction

The aerial parts (AE) and roots (R) of the *Seseli petraeum* (SP) were dried in the shade and powdered by using a laboratory-scale mill. The parts of the SP (each 30 g) were extracted by *n*-hexane, ethyl acetate (AcOEt), and methanol (MeOH) using the Soxhlet apparatus (x100 mL) for 3 hours, subsequently. Solvents were evaporated to dryness under reduced pressure at 40°C to obtain crude extracts (Table I). Then, six types of extracts in different polarities were obtained, as follows in Table I.

Cell culture and treatments

A549 human lung cancer cells were purchased from the American Type Cell Culture Collection

Table I. The amounts of extracts from the different polarity of solvents.

Plant Samples (30 g)	n-Hexane Extract (g)	Yield %	AcOEt Extract (g)	Yield %	MeOH Extract (g)	Yield %
Root	[1] 2.69 SPRH	8.96	[2] 1.42 SPRE	4.73	[3] 4.71 SPRM	15.7
Aerial part	[4] 2.50 SPAEH	9.33	[5] 0.91 SPAEE	3.03	[6] 5.45 SPAEM	18.16

[1]-SPRH: *Seseli petraeum* root hexane extract; [2]-SPRE: *Seseli petraeum* root ethyl acetate extract; [3]-SPRM: *Seseli petraeum* root methanol extract; [4]-SPAEH: *Seseli petraeum* aerial part hexane extract; [5]-SPAEE: *Seseli petraeum* aerial part ethyl acetate extract; [6]-SPAEM: *Seseli petraeum* aerial part methanol extract.

(ATCC, Germany). The cells were cultured in DMEM supplemented with 10% fetal bovine serum (Lonza, Germany) in 5% CO_2 incubator at 37°C. The cells were treated with 0.5, 1, 2.5, 5, and 10 mg/mL concentrations of the extracts from *Seseli petraeum* and incubated for 24 h under the same culture conditions. The stock solutions of extracts were dissolved in DMSO (Dimethyl sulfoxide), and the final concentration of this solvent was kept constant at 0.01%. The non-treated cells were used as control.

Cell growth assay

Different methods are used to screen anticancer agents. One of the techniques is known as MTT [3-(4,5-dimethylthiazol-2yl)-2,4-diphenyltetrazolium bromide] assay, which is a reliable and primary method for preliminary evaluation of anticancer agents (Mosmann 1983, Alley et al. 1988). The effect of the extracts on cellular growth of A549 cells was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole] assay. 180 µL of cell suspension from $5x10^4$ cells/mL were plated in a 96-well microplate. The cells were treated with 0.5, 1, 2.5, 5, and 10 mg/mL of the extracts for 24 h. Following incubation, the cells were treated with MTT solution (5 mg/mL) and incubated for 2 hours. The absorbance of dissolved formazan crystals was recorded at 540 nm by spectrophotometer (Thermo, Germany). Results are expressed as the mean±S.D. of three independent experiments. The differences are * $p<0.01$ and ** $p<0.0001$ compared to control. The IC_{50} value (The half-maximal inhibitory concentration) was calculated through linear regression analysis of cell proliferation results and given in Table II.

Cell cycle analysis

Muse Cell Cycle Assay Kit (Millipore, Germany) has been used to determine the effect of the extract on cell cycle arrest. A549 cells were plated on 12-well plates at a density of $2x10^5$ cells/well and

Table II. IC_{50} values of extracts determined by MTT assay.

Extracts	IC_{50} values (mg/mL)
[1]-SPRH	3.432
[2]-SPRE	5.660
[3]-SPRM	5.661
[4]-SPAEH	15.567
[5]-SPAEE	10.604
[6]-SPAEM	8.318

treated with hexane extract of the root (SPRH) at 3.432 mg/mL, which is previously determined as IC_{50} value of extract by MTT assay. Following incubation for 24 h, the cells were harvested and then fixed with 70% ethanol at 4°C for 4 h. Cell pellets were then collected by centrifugation at 400 x g for 10 min and incubated with assay solution for 30 min. The Muse Cell Analyzer (Millipore, Germany) has been used to detect three phases of the cell cycle, including G0/G1, S, and G2/M.

Annexin V binding assay

A549 cells were plated into six-well plates at a density of 1×10^6 cells per well and incubated for 24 hr. The cells were then treated with 3.432 mg/mL SPRH. Following incubation, the cells were harvested, and the Annexin V assay kit (Millipore) was performed according to the manufacturer's instructions. Briefly, 100 µl of Annexin V reagent added to 100 µl of cell suspension and incubated for 20 min at room temperature. Then, the apoptotic cell population was detected by Muse Cell Analyzer (Millipore). This assay utilizes Annexin V to detect PS on the external membrane of apoptotic cells. It is excluded from live, healthy cells, as well as early apoptotic cells. Four populations of cells can be distinguished in this assay: non-apoptotic cells: Annexin V (-) and 7-AAD (-); early apoptotic cells: Annexin V (+) and 7-AAD (-); late-stage apoptotic and dead cells: Annexin V (+) and 7-AAD (+); mostly nuclear debris: Annexin V (-) and 7-AAD (+).

Fluorescence imaging

The A549 cells were stained to visualize the effects of SPRH on apoptosis, using Annexin V-FITC and PI dye (BD, Germany). The cells were placed into an 8-well chamber slide (Millipore, Germany) at a density of 5×10^4 cells per well and incubated for 24 h. Then, the cells were treated with 3.432 mg/ml of SPRH and incubated for 24 h again. Following incubation of cells with the extract, cells were incubated with 20 µl of Annexin V-FITC and/or PI dye for 20 min at room temperature in the dark. Images were obtained using an inverted fluorescence microscope (Olympus, Germany).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 6.0 version (GraphPad Software Inc.). Data obtained from the cell culture experiments were expressed as mean ± SD, and a one-way ANOVA test was applied for multiple comparisons.

RESULTS

In the present study, the plant extracts were obtained by gradually increasing the polarity of solvents (hexane, ethyl acetate, and methanol) in a Soxhlet apparatus. The amounts and yields of extracts are given in Table I.

The cells have proceeded with different concentrations of *Seseli petraeum* extracts between 0.5 to 10 mg/mL for 24 hr, and their cytotoxic effect on A549 human lung cancer cells was evaluated by MTT test (Figure 1). Although our results showed that almost all parts (aerial parts and roots) of the SP had significant effects on the inhibition of cell proliferation, the hexane extract from the root [1]-SPRH was the most potent inhibitor of cell growth with an IC_{50} value of 3.432 mg/mL (Table II). The viable cell amount in [1]-SPRH treated group significantly decreased to 75.20±5.22% and 12.64±0.24% at 1 mg/mL and 2.5 mg/mL concentrations, respectively.

The further analyses were performed using extract [1]-SPRH at IC_{50} concentration. The cell population percent significantly increased to 11.70±1.50% in [1]-SPRH treated group, whereas it was 1.80±0.06% in the nontreated control group

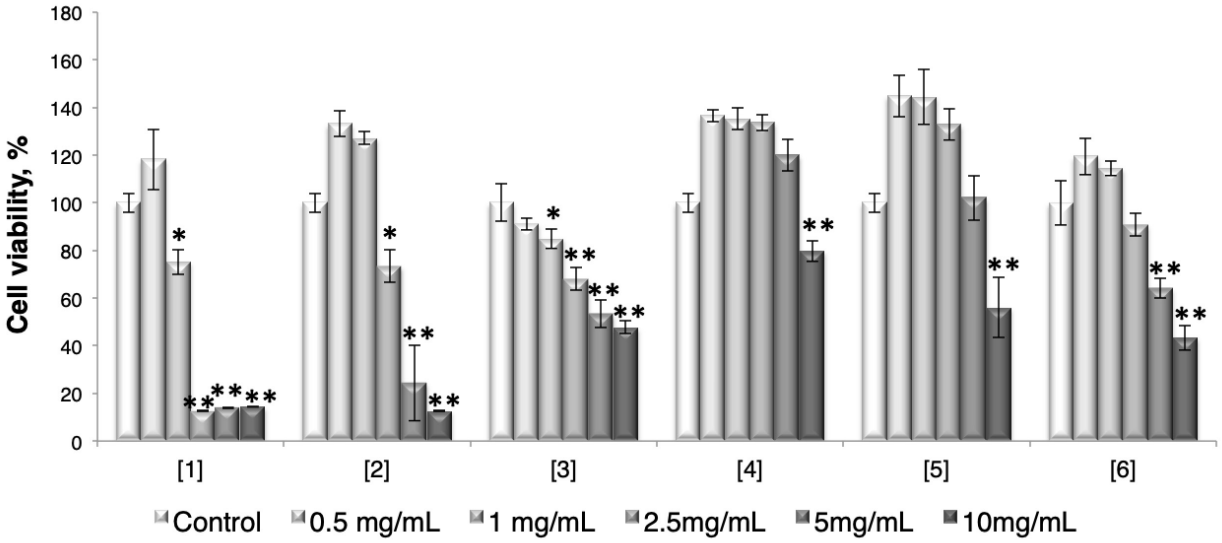

Figure 1. The effect of the extracts *Seseli petraeum* on cell growth of A549 human lung cancer cells. The 180 µl of A549 cell suspension from 5x10^4 cells/ml were plated in a 96-well microplate. A549 cells were exposed to different concentrations of extracts (0.5-10 mg/mL) for 24 h. Following incubation, the cells were treated with MTT [3-(4,5-dimethylthiazol-2yl)-2,4-diphenyltetrazolium bromide] solution (5 mg/mL) and incubated for 2 hours. The absorbance of dissolved formazan crystals was recorded at 540 nm by spectrophotometer (Thermo, Germany). Results are expressed as a percentage of viable cell amount. The non-treated cells were used as control. Each value represents the mean±standard deviation from three independent experiments performed in triplicate. (*p < 0.01, **p < 0.0001, compared to control). (The numbers define the extracts as; [1]-SPRH; [2]-SPRE; [3]-SPRM; [4]-SPAEH; [5]-SPAEE; [6]-SPAEM).

(p<0.01). The results of cell cycle analysis showed that the extract [1] has significantly induced a cell cycle arrest at the G0/G1 phase (Figure 2).

The effects of extract [1]-SPRH on apoptosis of A549 cells were evaluated by measuring the binding amount of annexin V to phosphatidylserine, which is exposed to cell surface an early marker of apoptosis (Figure 3). In extract [1]-SPRH treated group, the live cell population % significantly decreased to 70.66±1.58 % (p<0.05), while the population % of late apoptosis increased to 21.01±3.37 % (p<0.05). Through fluorescence imaging, it has also been determined that extract [1]-SPRH has induced DNA degradation strongly when compared to control (Figure 4).

Our results showed that, although all extracts prepared from aerial and roots of SP had notable effects on inhibition of cell proliferation, the hexane extract of the roots of this plant [1]-SPRH had a more potent effect compared to the other extracts obtained by the different polarity of the solvents.

DISCUSSION

Cancer is one of the leading causes of death and still maintain to be a significant health problem worldwide. The new anticancer agents from natural sources have yet been discovered in the scientific and commercial field (Parkin et al. 2005, Jemal et al. 2010) but still an urgent need for the development of new anticancer agents. Thousands of plants have been found to have significant anticancer effects (Mukherjee et al. 2001, Balunas & Kinghorn 2005, Cragg & Newman 2005, Srivastava et al. 2005). Since a long time, many studies have indicated that

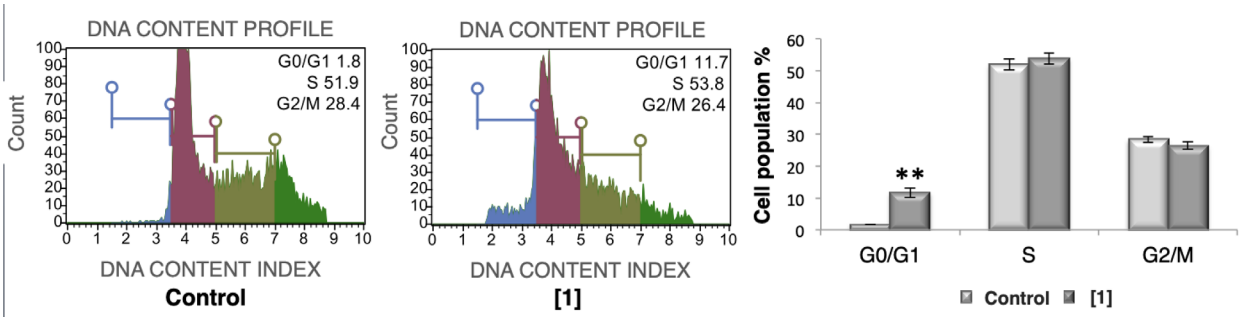

Figure 2. The results of cell cycle analysis. The A549 cells were plated on 12-well plates at a density of $2x10^5$ cells/well and treated with 3.432 mg/mL of hexane extract from the root [1] for 24 hr and cell cycle assay was performed according to the instructions as described in the methods section. Later, the cell population percent at different phases of the cell cycle, including G0/G1, S, and G2/M, were detected by Muse Cell Analyzer (Millipore, Germany). The results were given for three independent experiments. The differences are ** from control ($p<0.0001$).

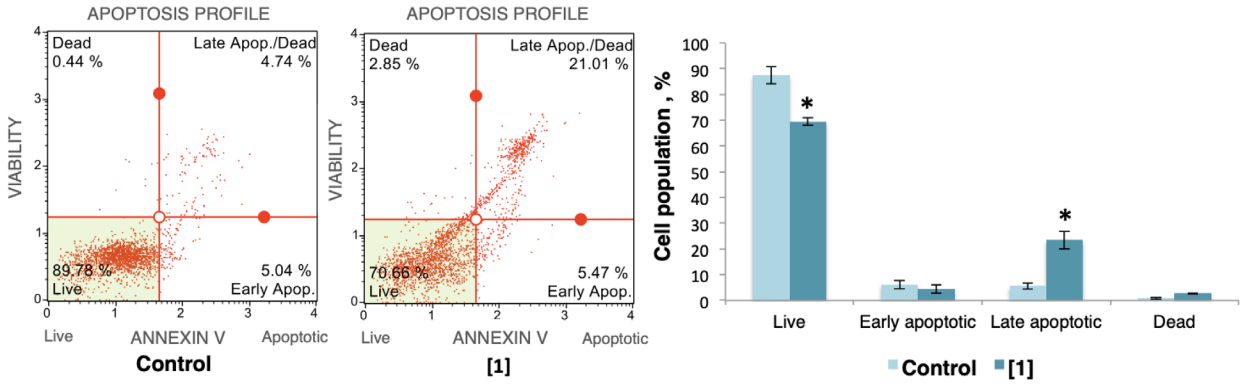

Figure 3. The results of the Annexin V binding assay. The cells were plated in a 12-well plate at a density of $2x10^5$ cells/ml and treated with 3.432 mg/mL of hexane extract from the root [1] for 24 hr, and the apoptosis was detected by the Muse cell analyzer (Millipore). Nontreated cells were used as control. The apoptotic cells were determined by the Annexin V positivity based on phosphatidylserine exposure, and dead cells were determined by the nuclear dye 7-AAD (7-aminoactinomycin D) positivity. Four different cell population were enabled to examine by cytofluorometric separation on a Muse cell analyzer: non-apoptotic live (lower left (LL): 7-AAD negative, apoptosis negative), non-apoptotic dead (upper left (UL): 7-AAD positive, apoptosis negative), apoptotic live (lower right (LR): 7-AAD negative, apoptosis-positive), and apoptotic dead (upper right (UR):7-AAD positive, apoptosis-positive) cells. The results were given for three independent experiments, and the differences are * from control ($p<0.01$).

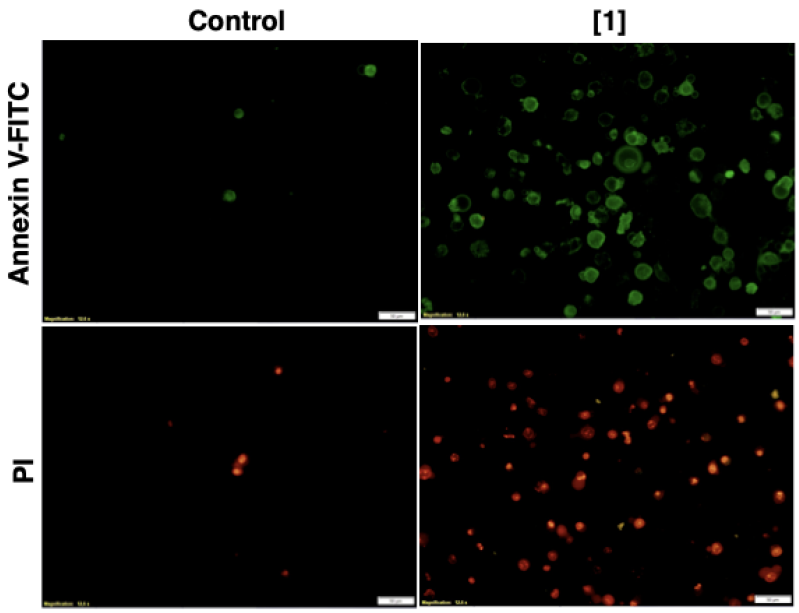

Figure 4. Fluorescence microscopy analysis of Annexin V-FITC staining. A549 lung cancer cells were seeded into an 8-well chamber slide (Millipore, Germany) at a density of 5×10^4 cells per well and incubated for 24 h. Following incubation, the cells were left untreated (control) and treated with 3.432 mg/mL of hexane extract from the root [1] for 24 hr, and the fluorescence images were obtained by Annexin V and propidium iodide staining protocol according to the instructions (BD Biosciences, Germany) through fluorescence microscope (Olympus, Germany). The results were imaged for three independent experiments.

the mechanism of action of many anticancer drugs is based on apoptosis induction, and thus opening a new strategy in search of anti-cancer drugs (Cohen et al. 1997, Hanahan & Weinberg 2000, Sun et al. 2004, Wong 2011, Ramasamy et al. 2012).

Despite the high potential of *Seseli* species to be a valuable source of bioactive molecules, not enough studies have been done on chemical and biological activity. The literature revealed that *Seseli* species have mainly included coumarins (Barrero et al. 1990, Cecherelli et al. 1990, Glowniak et al. 1991, Tosun et al. 2005), cinnamic acid derivatives (Banerjee et al. 1987), sesquiterpene lactones, phenylpropanoids (Barrero et al. 1994) and essential oil (Bader et al. 2003, Habibi et al. 2003, Kaya et al. 2003). However, Vuckovic´ et al. (2010) have proved that a prenylated flavanonol named seselinonol from *S. annuum* L. roots exhibited a beneficial effect by decreasing DNA damage of human lymphocytes using cytochalasin-B blocked micronucleus (CBMN) assay (Vuckovic´ et al. 2010). Moreover, a tetrahydrofuranoid lignan named seselinone and eudesmin from the aerial parts of *S. anuum* L. showed cytotoxic activity against C6 rat glioma cell cultures in a different study performed by the same research group (Vuckovic´ et al. 2007). When the cytotoxic activity of the essential oils of *Seseli tortuosum* and *S. montanum* subsp. *peixotoanum* compared; it was observed that *S. tortuosum* essential oil has cytotoxic properties to human cells for the assessment of keratinocytes viability when used in concentrations higher than 0.64 µL/mL by MTT assay (Gonçalves et al. 2012). The ethanolic extract of the roots of *S. mairei* Wolf. have exhibited notable cytotoxic effects against KB, P-388, L-1210 tumor cell lines (ED50 < 20 µg/mL). Later, it was understood that this effect was caused by seselidiol, a polyacetylene derivative in the active fraction (Hu et al. 1990). In our study, we observed a significant effect on hexane extract of the roots of *S. petraeum* due to the nonpolar compounds (nonpolar coumarins) mostly pass through the hexane, and the result may depend on it.

Moreover, some of the pyranocoumarin derivatives called as 3',4'-dihydroxy-3',4'-dihydroseselin are also exhibited cytotoxic effects against P-388 lymphocytic leukemia cells (Egan et al. 1990) besides the many other coumarin derivatives which are displayed anticancer potential (Kaur et al. 2015, Venugopala et al. 2013). In a previous study, the pyranocoumarin-type coumarins have been isolated from the hexane extract of *Seseli gummiferum* ssp. *corymbosum* (Tosun et al. 2005, 2007, Tosun 2006). Moreover, one of the pyranocoumarin called corymbocoumarin isolated hexane extract of this plant has been found active for lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages, and consider that it could be a useful therapeutic approach for inflammation-associated diseases (Chun et al., 2016). The hexane extracts of not only *Seseli* species but also other species had been found active against some of the cancer cell lines. For example, the hexane extract of the peels of *Citrus hystrix* (Rutaceae) fruits exhibited preferential cytotoxicity against PANC-1 (human pancreatic cancer cells) using a nutrient-deprived medium. This bioactive extract has furanocoumarins, and simple coumarins, which were tested for their preferential cytotoxicity against three different human pancreatic cancer cell lines (PANC-1, MIA PaCa-2, and PSN-1), and bergamottin was found the most active furanocoumarin (Sun et al. 2018). The bioassay-guided fractionation of the *n*-hexane extract of *Citrus reticulata* Blanco stem bark led to several coumarins and evaluated using MTT assay against three human cancer cell lines (human lung adenocarcinoma cell line A549, human breast adenocarcinoma cell line MCF7, and human Caucasian prostate adenocarcinoma cell line PC3). A significant activity of the *n*-hexane (IC_{50}= 45.6 µg/mL) and the dichloromethane extracts (IC_{50}= 54.7 µg/mL) have been found against the breast cancer cell line MCF7 (Tahsin et al. 2017).

The present study has demonstrated that the extracts of *Seseli petraeum* have a potent antiproliferative effect against A549 lung cancer cells through induction of cell cycle arrest at G0/G1 phase and within the lowest IC_{50} value, the hexane extract from the root codded as [1]-SPRH has significantly induced apoptosis of A549 cells as well as DNA damage. Further analyses will be helpful to identify the mechanisms underlying these effects. Furthermore, this effect could be attributed to coumarins passing towards the non-polar solvent (like hexane), which is usually present in the aglycone structure.

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REFERENCES

AKALIN URUŞAK E & KIZILARSLAN Ç. 2013. Fruit anatomy of some *Ferulago* (Apiaceeae) species in Turkey. Turk J Bot 37: 434-445.

ALLEY MC, SCUDIERO DA, MONKES A, HURSEY ML, CZERWINSKI MJ, FINE DL, ABBOTT BJ, MAYO JG, SHOEMAKER RH & BOYD MR. 1988. Feasibility of drug screening with panel of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 48: 589-601.

AYTAÇ Z & DUMAN H. 2013. A new species and two new records from Turkey. Turk J Bot 37: 1055-1060.

BADER A, CAPONI S, CIONI PL, GUIDO F & MORELLI I. 2003. Acorenone in the essential oil of flowering aerial parts of *Seseli tortuosum* L. Flavour Fragr J 18: 57-58.

BALUNAS MJ & KINGHORN AD. 2005. Drug discovery from medicinal plants. Life Sci 78(5): 431-441.

BANERJEE SK, MUKHOPADHYAY S, GUPTA BD, SINGH K & RAJ S. 1987. Sesebrinic acid, a cinnamic acid derivate from *Seseli sibiricum*. Phytochemistry 26: 1817-1820.

BARRERO AF, HERRADOR MM & ARTEAGA P. 1990. Cumarinas en especies del genero *Seseli* (Fam. Umbelliferae). Ars Pharm 31(3-4): 241-256.

BARRERO AF, HERRADOR MM & ARTEAGA P. 1994. Sesquiterpene lactones and other constituents of *Seseli vayredanum*. Phytochemistry 37: 1351-1358.

BAYTOP T. 1994. Türkçe Bitki Adları Sözlüğü. Atatürk Kültür, Dil ve Tarih Yüksek Kurumu, TDKY 3578, Ankara, TTK Basımevi, p. 169.

BAYTOP T. 1999. Türkiye'de Bitkiler ile Tedavi. 2. Baskı, Ankara, Nobel Tıp Kitapevi, p. 375.

CANTER PH, THOMAS H & ERNST E. 2005. Bringing medicinal plant species into cultivation: opportunities and challenges for biotechnology. Trends Biotechnol 23: 180-185.

CECHERELLI P, CURINI M, MARCOTULLIO MC & MADRUZZA G. 1990. Tortuoside, a new natural coumarin glucoside from *Seseli tortuosum*. J Nat Prod 53(2): 536-538.

CETIN O, SEKER MO & DURAN A. 2015. A new subspecies of *Seseli gummiferum* (Apiaceae) from Ilgaz Mountain National Park, northern Turkey. PhytoKeys 56: 99-110.

CHUN J, TOSUN A & KIM YS. 2016. Anti-inflammatory effect of corymbocoumarin from *Seseli gummiferum* subsp. *corymbosum* through suppression of NF-κB signaling pathway and induction of HO-1 expression in LPS-stimulated RAW 264.7 cells. Int Immun 31: 207-215.

COHEN GM. 1997. Caspases: the executioners of apoptosis. Biochem J 326: 1-16.

CRAGG GM & NEWMAN DJ. 2005. Plants as a source of anti-cancer agents. J Ethnopharmacol 100: 62-79.

CROWDEN RK, HARBORNE JB & HEYWOOD VH. 1969. Chemocystematics of the Umbelliferae-A general survey. Phytochemistry 8: 1963-1984.

DAVIS PH, MILL RR & TAN K. 1988. Flora of Turkey and The East Aegean Islands. Edinburgh, Edinburgh University Press Vol 10: 265-267.

DIOSCORIDES P. 2002. De Materia Medica. Athens. Militos Press, p. 215.

DUMAN H. 2000. *Seseli* L. In Flora of Turkey and The East Aegean Islands, Ed.: Güner A, Özhatay N, Ekim T, Başer KHC, Edinburgh, Edinburgh University Press Vol 11, p. 141.

EGAN D, O'KENNEDY R, MORAN E, COX D, PROSSER E & THORNES RD. 1990. The pharmacology, metabolism, analysis, and applications of coumarin and coumarin-related compounds. Drug Metab Rev 22(5): 503-529.

GLOWNIAK K, DORACZYNSKA-SZOPA A, ERKELENS C & VAN DER SLUIS WG. 1991. Isopeucenidin and libanotin from *Libanotis intermedia* roots: Isolatin and NMR-analysis. Planta Med 57(Suppl 2): A 52.

GONÇALVES MJ, TAVARES AC, CAVALEIRO C, CRUZ MT, LOPES MC, CANHOTO J & SALGUEIRO L. 2012. Composition, antifungal activity and cytotoxicity of the essential oils of *Seseli tortuosum* L. and *Seseli montanum* subsp. *peixotoanum* (Samp.) M. Laínz from Portugal. Ind Crops Prod 39: 204-209.

GUNER E, DUMAN H & PINAR N. 2011. Pollen morphology of the genus *Seseli* L. (Umbelliferae) in Turkey. Turk J Bot 35: 175-182.

GUNER E & DUMAN H. 2013. The revision of genus *Seseli* (Umbelliferae) in Turkey. Turk J Bot 37: 1018-1037.

HABIBI Z, MASOUDI S & RUSTAIYAN A. 2003. Chemical composition of the essential oil of *Seseli tortuosum* L. ssp. *kiabii* Akhani. from Iran. J Essent Oil Res 15: 412-413.

HAMLYN P. 1969. In: The Marshall Cavendish, Encyclopedia of Gardening. Garrod and Lofthouse International, London, 19, p. 2034.

HANAHAN D & WEINBERG RA. 2000. The hallmarks of cancer. Cell 100: 57-70.

HEDGE IC & LAMOND JM. 1972. *Seseli* L. In Flora of Turkey and The East Aegean Islands Ed.: P.H. Davis, Edinburgh, Edinburgh University Press Vol 4: 367-372.

HEYWOOD VH, BRUMMITT RK, CULHAM A & SEBERG O. 2007. Apiaceae. In: Flowering Plant Families of the World. New York, Firefly Books p. 35-38.

HEYWOOD VH. 1979. Flowering plants of the World. Oxford, Oxford University Press. Hochmuth DH, MassFinder 4.0, Hochmuth Scientific Consulting, Hamburg, Germany, p. 219.

HU CQ, CHANG JJ & LEE KH. 1990. Antitumor agents, 115. Seselidiol, A new cytotoxic polyacetylene from *Seseli mairei*. J Nat Prod 53: 932-935.

ILIC MD, STANKOV JOVANOVIC VP, MITIC VD, JOVANOVIC OP, MIHAJILOV-KRSTEV TM, MARKOVIC MS & STOJANOVIC GS. 2015. Comparison of chemical composition and biological activities of *Seseli rigidum* fruit essential oils from Serbia. Open Chem 13: 42-51.

JEMAL A, SIEGEL R, XU J & WARD E. 2010. Cancer statistics 2010. Cancer J Clin 60: 277-300.

KAUR M, KOHLI S, SANDHU S, BANSAL Y & BANSAL G. 2015. Coumarin: a promising scaffold for anticancer agents. Anticancer Agents Med Chem 15(8): 1032-1048.

KAYA A, DEMIRCI B & BASER KHC. 2003. The essential oil of *Seseli tortuosum* L. growing in Turkey. Flavour Fragr J 18: 159-161.

KHAN S, SHEHZAD O, CHENG MS, LI RJ & KIM YS. 2015. Pharmacological mechanism underlying anti-inflammatory properties of two structurally divergent coumarins through the inhibition of pro-inflammatory enzymes and cytokines. J Inflamm 12: 47.

KUPELI E, TOSUN A & YESILADA E. 2006. Anti-inflammatory and antinociceptive activities of *Seseli* L. species (Apiaceae) growing in Turkey. J Ethnopharmacol 104: 310-314.

LYSKOV D, DOGAN GUNER E, SAMIGULLIN T, DUMAN H & PIMENOV M. 2018. Molecular data to elucidate taxonomy in *Seseli* sect. *Seseli* (Apiaceae) of East Mediterranean and Southern Europe. Nord J Bot 36: 2-7.

MATEJIC SJ, DZAMIC AM, MIHAJILOV-KRSTEV T, RANDELOVIC VN, KRIVOSEJ ZD & MARIN PD. 2012. Total phenolic content, flavonoid concentration, antioxidant and antimicrobial activity of methanol extract from three *Seseli* L. taxa. Cent. Eur J Biol 7(6): 1116-1122.

MOSMANN T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J lmmunol Methods 65: 55-63.

MUKHERJEE AK, BASU S, SARKAR N & GHOSH AC. 2001. Advances in cancer therapy with plant-based natural products. Curr Med Chem 8: 1467-1486.

NEWMAN DJ & CRAGG GM. 2016. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 79(3): 629-661.

OZHATAY N, AKALIN E, OZHATAY E & UNLU S. 2009. Rare and endemic taxa of Apiaceae in Turkey and their conservation significance. J Fac Pharm Istanbul 40: 2-15.

OZTURK A, OZTURK S & KARTAL S. 2000. The characteristics and uses of herbs added to herby cheeses in Van. Herb J Syst Bot 7: 167-181.

OZTURK S & ERCISLI S. 2006. Chemical composition and in vitro antibacterial activity of *Seseli libanotis*. World J Microbiol Biotechnol 22: 261-264.

PARKIN DM, BRAY F, FERLAY J & PISANI P. 2005. Global cancer statistics. CA Cancer J Clin 55: 74-108.

PIMENOV MG & LEONOV MV. 1993. The Genera of The Umbelliferae. Whitstable Litho, Whitstable, Kent, Great Britain, p. 156.

PIMENOV MG & LEONOV MV. 2004. The Asian Umbelliferae biodiversity database (ASIUM) with particular reference to South-West Asian taxa. Turk J Bot 28: 139-145.

RAMASAMY S, WAHAB NA, ABIDIN NZ, MANICKAM S & ZAKARIA Z. 2012. Growth inhibition of human gynecologic and colon cancer cells by *Phyllanthus watsonii* through apoptosis induction. PLoS ONE 7(4): e34793.

SAYED-AHMAD B, TALOU T, SAAD Z, HIJAZI A & MERAH O. 2017. The Apiaceae: ethnomedicinal family as a source for industrial uses. Ind Crops Prod 109: 661-671.

SINGH G, KAPOOR IP, PANDEY SK, SINGH UK & SINGH RK. 2002. Studies on essential oils: antibacterial activity of volatile oils of some spices. Phytother Res 16(7): 680-682.

SINGH T & SINGH A. 2011. A review on natural products as wood protectant. Wood Sci Technol 46(5): 1-17.

SRIVASTAVA V, NEGI AS, KUMAR JK, GUPTA MM & KHANUJA SP. 2005. Plant-based anticancer molecules: a chemical and biological profile of some important leads. Bioorg Med Chem 13: 5892-5908.

STANKOV-JOVANOVIC VP, ILIC MD, MITIC VD, MIHAJILOV-KRSTEV TM, SIMONOVIC SR, NIKOLIC MANDIC SD, TABET JC & COLE RB. 2015. Secondary metabolites of *Seseli rigidum*: Chemical composition plus antioxidant, antimicrobial and cholinesterase inhibition activity. J Pharm Biomed Anal 111: 78-90.

STOJKOVIC S, PETROVIC S, KUKIC J, DZAMIC A, RISTIC M, MILENKOVIC M, GLAMOCLIJA J, SOKOVIC M & STAJKOVIC D. 2009. Chemical composition and antimicrobial and antioxidant activity of *Seseli rigidum* flower essential oil. Chem Nat Comp 45: 2.

SUN SY, HAIL N & LOTAN JRR. 2004. Apoptosis as a novel target for cancer chemoprevention. J Natl Cancer Inst 96: 662-672.

SUN S, PHRUTIVORAPONGKUL A, DIBWE DF, BALACHANDRAN C & AWALE S. 2018. Chemical constituents of Thai *Citrus hystrix* and their antiausterity activity against the PANC-1 human pancreatic cancer cell line. J Nat Prod 81: 1877-1883.

TAHSIN T, DUPLEX WANSI J, AL-GROSHI A, ANDREW EVANS A, NAHAR L, MARTIN C & SARKER SD. 2017. Cytotoxic properties of the stem bark of *Citrus reticulata* Blanco (Rutaceae). Phytother Res 31: 1215-1219.

TOSUN A. 2006. Occurrence of coumarins in *Seseli hartvigii* growing in Turkey. Chem Nat Compd 42: 608-609.

TOSUN A, BAHADIR Ö & DİNÇ E. 2007. Determination of anomalin and deltoin in *Seseli resinosum* by LC combined with chemometric methods. Chromatographia 66: 677-683.

TOSUN A, CHUN J, JERKOVIC I, MARIJANOVIC Z, FENU MA, ASLAN SS, TUBEROSO CIG & KIM YS. 2016. Chemical profiles and anti-inflammatory activity of the essential oils from *Seseli gummiferum* and *Seseli corymbosum* subsp. *corymbosum*. Nat Prod Commun 11(10): 1523-1526.

TOSUN A, KURKCUOGLU M, DOGAN E, DUMAN H & BASER KHC. 2006. Essential oil composition of *Seseli petraeum* M. Bieb. and *Seseli andronakii* Woron. growing in Turkey. Flavour Fragr J 21(2): 257-259.

TOSUN A, OZKAL N, BABA M & OKUYAMA T. 2005. Pyranocoumarins from *Seseli gummiferum* subsp. *corymbosum* growing in Turkey. Turk J Chem 29: 327-334.

VENUGOPALA KN, RASHMI V & ODHAV B. 2013. Review on natural coumarin lead compounds for their pharmacological activity. Biomed Res Int 963248.

VUCKOVIC I, TRAJKOVIC V, MACURA S, TESEVIC V, JANACKOVIC P & MILOSAVLJEVIC S. 2007. A novel cytotoxic lignan from *Seseli annuum* L. Phytother Res 21(8): 790-792.

VUCKOVIC I, VAJS V, STANKOVIC M, TESEVIC V & MILOSAVLJEVIC S. 2010. A new prenylated flavanonol from *Seseli annuum* roots showing protective effect on human lymphocytes DNA. Chem & Bioadv 7(3): 698-704.

WONG RSY. 2011. Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res 30: 87-90.

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