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Anti-Cancer Activity of *Rhododendron luteum* Flower Extracts on Non-Transformed and Transformed Cell Lines

Saliha Ekşi[1*], Nebahat Ejder[1], Osman Birol Özgümüş[1], Barış Uzunok[2] and Vagıf Atamov[3]

*[*1]Department of Medical Microbiology, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize, Turkey*

[2]Department of Medical Physiology, Faculty of Medicine, Uşak University, Uşak, Turkey

[3]Department of Botanica, Faculty of Arts and Sciences, Recep Tayyip Erdoğan University, Rize, Turkey.

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ABSTRACT

This study aimed to determine the anti-proliferative properties of different solvent extracts obtained from various parts of *Rhododendron luteum*. In order to investigate anti-cancer activity, non-transformed and transformed cell lines were treated by various organic extracts (ethyl acetate, hexane and methanol) obtained from different flower parts of *R. luteum* or DMSO for 48 h in *in vitro* conditions. Cytotoxic activity measurement was achieved by the MTT method. The results obtained from this study showed that ethyl acetate extracts derived from the sepals, petals and buds illustrated significant anti-proliferative effects on the HT29 colorectal cancer cell line. The IC_{50} values that were determined for sepal, petal and bud ethyl acetate extracts were 133.2 µg/ml, 459.3 µg/ml and 159.94 µg/ml, respectively in comparison to the ARPE-19 IC_{50} values which were 266.6 µg/mL, 800 µg/ml and 366.65 µg/ml, respectively. The difference between the non-transformed ARPE-19 and transformed HT-29 cell lines was statistically significant ($p<0.05$). In consequence, the results from this study suggest that different organic extracts from *R. luteum* may have selective effects on different cancer cells and offer a potential for usage as anticancer agents for colorectal cancer. For determination of this potential, further studies are needed to assess the activity of the compound/compounds that may mediate selective effects on different types of cancer cells.

Keywords: *R. luteum*, Cancer, Cytotoxicity, MTT, HT-29, HeLa, A549, ARPE-19

INTRODUCTION

Cancer is a growing global public health problem that leads to significant morbidity and mortality every year[1-3]. Cancer is a systemic disease which emerges when changes occur in normal cells and spreads into the surrounding normal tissue or throughout the whole body. Therefore, it is thought that chemotherapy is the most effective treatment. However, the fact that drugs that are used in chemotherapy are also effective on normal cells and, more importantly, the ability of cancer cells to develop resistance to these drugs over time [4-6] have led scientists to investigate new anticancer agents.

Natural products have been used traditionally for more than 5.000 years and even today more than 60% of the world's population depends on medicinal plants as their primary source of healthcare [7,8]. Among herbal and plant medicines, Rhododendrons (Ericaceae) have gained prominence in recent years for their secondary metabolites [9-14] and anti-carcinogenic potential [15,16]. Rhododendron is one of the largest genera of plants; it is a small, woody, evergreen shrub, mainly distributed on the Northern hemisphere and have been widely used for traditional medicine in China and Korea [17].

The first written reference about Rhododendron dates back as far as 401 B.C. and records the toxicity of rhododendron honey [18]. Some *Rhododendron* spp. still known to cause intoxication, mainly due to grayanotoxin-contaminated honey [19,20]. To the best of our knowledge, biological research on the genus goes back to 1960. *Rhododendron* spp. are a rich source of secondary metabolites and some of these induce various kinds of bioactivities [9] which promise therapeutic potential. Recently, antimicrobial [14], anti-

Corresponding author: Saliha Ekşi, Assistan Professor of Microbiology, Department of Medical Microbiology, School of Medicine, R.T Erdogan University, 53100 Rize, Turkey, E-mail: saliha.eksi@erdogan.edu.tr.com

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protozoal [21], antioxidant [9-11] and anti-inflammatory [9-22] properties of this plant have been shown. In this study, we examined the cytotoxic effects of Rhododendron flower extracts on different types of cancer and the human normal cell lines *in vitro* conditions. As a result, we demonstrated that different solvent extracts from Rhododendron flowers induced selective cytotoxic effects on different types of cancer cell lines.

MATERIALS AND METHODS

Reagents

All chemicals and reagents were procured from certified suppliers. DMSO (dimethyl sulfoxide), MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Taxol, D-PBS and trypan blue (0.4%) were purchased from Sigma Aldrich. RPMI-1640 (Hyclone), fetal bovine serum (FBS), antibiotics (100 µg/ml streptomycin + 100 U/mL penicillin), 0.25% trypsin-EDTA and Dulbecco's modified Eagle's medium (DMEM) were obtained from GIBCO. The ethyl acetate was supplied by Sigma-Aldrich (St. Louis, MO, USA), while methanol and hexane that were used for the exractions were obtained from Merck KGaA, Darmstandt, Germany).

Cell lines and cell culture

Adenocarcinomic human alveolar basal epithelial cell line A-549 and human cervical cancer epithelial cell line (HeLa) were kindly provided by Prof. Fikrettin Sahin (Yeditepe University, Istanbul, Turkey), human endometrial adenocarcinoma cell line CRL-2923 was a gift from Prof. Bedia Agachan Cakmaoglu (Istanbul University, Istanbul; Turkey) and diploid ARPE-19 retinal pigment epithelial cell line was kindly provided by Dr. Muradiye Acar (Turgut Ozal University, Ankara, Turkey). Human colon adenocarcinoma cell line HT-29 was obtained from the American Type Culture Collection (ATCC). The cell lines were maintained in RPMI-1640 or DMEM with 10% fetal bovine serum (FBS) and antibiotics (100 µg/mL streptomycin + 100 U/mL penicillin) in T25 flasks at 37°C in a humidified atmosphere of 5% CO_2. At 70-80% confluency, the cells were passaged enzymatically with 0.25% trypsin and sub-cultured in 25 cm^2 plastic flasks for further maintenance. The culture media were replaced every 2 days.

Samples and extraction

The flowers of *R. luteum* were collected from Ayder in Rize, Turkey in the month of April 2016. The plant species was identified by Prof. Vagif Atamov, at the Department of Biology, Recep Tayyip Erdogan University in Rize, Turkey. After identification, *R. luteum* flowers were separated into their parts (sepals, petals, buds) and the freshly collected specimens were stored at (-20°C) at the Microbiology Cell Culture Laboratory. Extractions were performed with the maceration method. In summary, the frozen samples of *R. luteum* were weighed (10-20 g) and ground into powder. Crude extracts were prepared by suspending the powders in 100 mL of, ethyl acetate (EtOAc), hexane and methanol (MeOH) and macerated in a shaker for 48 h at room temperature. Thereafter, the extracts were filtered through a Whatman paper and the filtrates were then concentrated using a rotary evaporator (LabTech.EV311) at 40°C. First of all, stock solutions of the extracts (50-200 mg/mL) were prepared in DMSO and all the extracts were stored at –20°C for further anti-proliferative activity testing. For all the experiments, the working solutions were prepared by diluting the stocks of *R. luteum* extracts in complete media to the desired concentrations immediately before use. The final DMSO (as a negative control) concentration during the assays was kept below 0.4%.

Morphological studies

Morphological studies were performed as described earlier by Eksi et al. [23]. Briefly, different volumes of each extract were added to the complete media in order to generate working solutions. Transformed (Hela, HT-29, A549, CRL2923) (1×10^4 cells/ml) and non-transformed ARPE cells (2×10^4 cells/ml) were seeded into 24-well culture plates with 1000 µL of growth media. After overnight cultivation, various concentrations of the test samples or solely the corresponding DMSO (max. 0.4%) alone were added to the plate wells. Taxol (5 nM) was used as a positive control. The cultures were cultivated at 37°C for 24 h in an atmosphere of 5% CO_2 and 95% air in an incubator. Afterwards, cellular morphology was viewed an under inverted microscope using a 10X magnification (Olympus CKX410). The changes in cellular morphology were photographed using a digital microscope camera (Olympus SC30).

Cytotoxic studies using MTT assay

Cytotoxic activity was evaluated based on the method described by Mosmann [24], with minor modifications. Briefly, after trypsinization, for the transformed cells, 1×10^3 cells/well were seeded into a flat-bottom 96-well microtiter plates in 100 µL of growth medium in duplicatse and allowed to adhere overnight. On the other hand, the cell count for the non-transformed diploid cells was adjusted to 2×10^3 cells/well. The next day, varying concentrations of extracts from the stocks were added to the microtiter plate wells in duplicates per concentrations, and serial dilutions ranging from (800 µg/mL to 15.6 µg/mL) were made in the plates. The negative control received the corresponding amount of DMSO alone, and all plates were incubated for 48 h at 37°C in 5% CO_2. After the exposure time, 10 µl of (5 mg/ml in water) filtered, sterilized MTT solution was added to each well and the cells were incubated for an additional 4 h at 37°C. Afterwards, the medium was removed, and the formazan crystals that formed in the viable cells during the MTT treatment were dissolved by adding 100 µl of DMSO per well. The plates were incubated at 37°C for 20 more

minutes to allow complete solubilization. Absorbance was then measured at 570 nm using an ELISA microplate reader (Thermo, Multiskan Go). All experiments were performed in duplicates and repeated at least three times. Growth inhibition was calculated using the following formula: % Growth inhibition = [(negative control OD–Sample OD)/Negative control ODl] × 100.

The cytotoxic concentrations of the extracts (IC_{50}) were calculated from the dose-response curve, which inhibited cell growth by 50%. The selective index (SI) was calculated from the ratio of the IC_{50} of the normal cells over the cancer cells. Extracts with SI values close to 3 were considered to have better biological activity against tumor cells than the normal cells in *in vitro* conditions [25].

Apoptosis studies

Gel electrophoresis: DNA fragmentation analysis was performed as follows: Transformed (Hela, HT-29, A549, CRL2923) (1×10^4 cells/ml) and non-transformed ARPE-19 cells (2×10^4 cells/ml) were seeded into 6-well culture plates in 2000 µl of growth medium. After incubation overnight, various concentrations of test samples or the solely the corresponding DMSO (max. 0.4%) alone were added to the wells. Taxol (5 nM) was used as a positive control as it induces DNA fragmentation. The treated and untreated cultures were incubated at 37°C for 48 h in an atmosphere of 5% CO_2 and 95% air in an incubator. After trypsinization, the cells were counted and centrifuged at 2000 rpm for 5 min. The harvested cells were rinsed twice in cold phosphate-buffered saline (PBS, pH 7.4). The supernatants were discarded and the pellets were used for genomic DNA isolation. DNA extraction was performed according to the manufacturer's instructions from a Wizard Genomic DNA Purification Kit (Promega, A1120) and stored at -20°C until needed. The DNA samples that were obtained from the cells and Thermo 1 Kb DNA molecular weight marker were size-fractionated in 1% agarose gel and visualized by ethidium bromide (EtBr) staining. The scanned images were processed using Bio-Imaging Systems (MiniLumi).

Hoechst (H33342) staining: Transformed (1×10^4 cells/ml) and non-transformed cells (2×10^4 cells/ml) were seeded into 6-well culture plates in 2000 µl of growth medium. After 24 h extract and controls treatment, the cells were trypsinized, washed with cold 1x PBS two times and fixed in cold (-20°C) MeOH for 5 min. A staining solution of Hoechst (H33342) was prepared immediately before use. The cells were then incubated with Hoechst (2 µg/ml) for 10 min and washed with cold 1x PBS two times. The nuclear morphology of the cells was viewed under a microscope using a 20x magnification (Leica fluorescence DM4000) and the results were photographed using a digital microscope camera (Leica DFC425).

STATISTICAL ANALYSIS

All experiments were performed at least three times and growth inhibition was calculated in terms of percentage by the formula: % Growth inhibition = [(negative control OD – Sample OD)/Negative control ODl] × 100. The statistical analysis of all data was performing using an unpaired *t*-test. A level of $p<0.05$ denoted significance in all cases.

RESULTS

Cytotoxicity

Cytotoxicity of sepal extracts (Hexane, MeOH, EtOAc): To evaluate the growth inhibition on non-transformed and transformed cell lines induced by the exracts from *R. luteum* sepals, cells were incubated in six different concentrations of hexane extract (between 300 and 9.3 µg/ml) or a carrier DMSO (negative control) alone in *in vitro* conditions. Following a 48 h treatment with the extract, cytotoxicity was determined by an MTT assay. As shown in **Figure 1A**, the hexane extract from the sepals had a dose-dependent antiproliferative effect on all the cell lines and there was no statistically significant difference between non-transformed and transformed cells in the terms of cytotoxicity.

The addition of various concentrations (between 400 and 35 µg/ml) of the sepal MeOH extract to the cell culture media exhibited no anti-proliferative effects on non-transformed ARPE-19 cells following 48 h treatment **(Figure 1B)** in *in vitro* conditions. Meanwhile, it was found to be more prominent in HeLa and HT-29 cancer cells in comparison to A549 and CRL-2923 cancer cell lines. It was determined that 400 µg/mL of the MeOH extract induced >60% and 52% cell death in the HeLa and HT-29 cell lines, respectively. In terms of cytotoxicity, the ARPE-19 cells were significantly less sensitive than the HT-29 and HeLa cells as represented by the calculated P values of P=0.025 and P=0.05, respectively.

Furthermore, the anticancer properties of the EtOAc extract obtained from the sepals of *R. luteum* were investigated. An MTT assay was performed with various concentrations (between 600 and 79 µg/ml) of the EtOAc extract with an exposure time of 48 h in *in vitro* conditions. As shown in **Figure 1C**, at high concentrations (≥ 400 µg/ml), the extract was highly cytotoxic and led to >60% cell growth inhibition in all the cells. However, as the concentrations of the extract gradually decreased to 177.7 µg/ml and 118.5 µg/ml), the transformed HT-29 cell line was more sensitive to the treatment by the EtOAc extract. In terms of cytotoxicity, the non-transformed ARPE-19 cells were significantly less sensitive than the HT-29 colorectal cancer cells ($p<0.05$). Additionally, the IC_{50} of EtOAc extract was determined to be 266.6 µg/ml for the non-transformed ARPE-19 cells. On the other hand, the lowest IC_{50} value (133 µg/ml) was calculated for HT-29 cells suggesting that the extract may have selective cytotoxic activity against the HT-29 colon cancer cells.

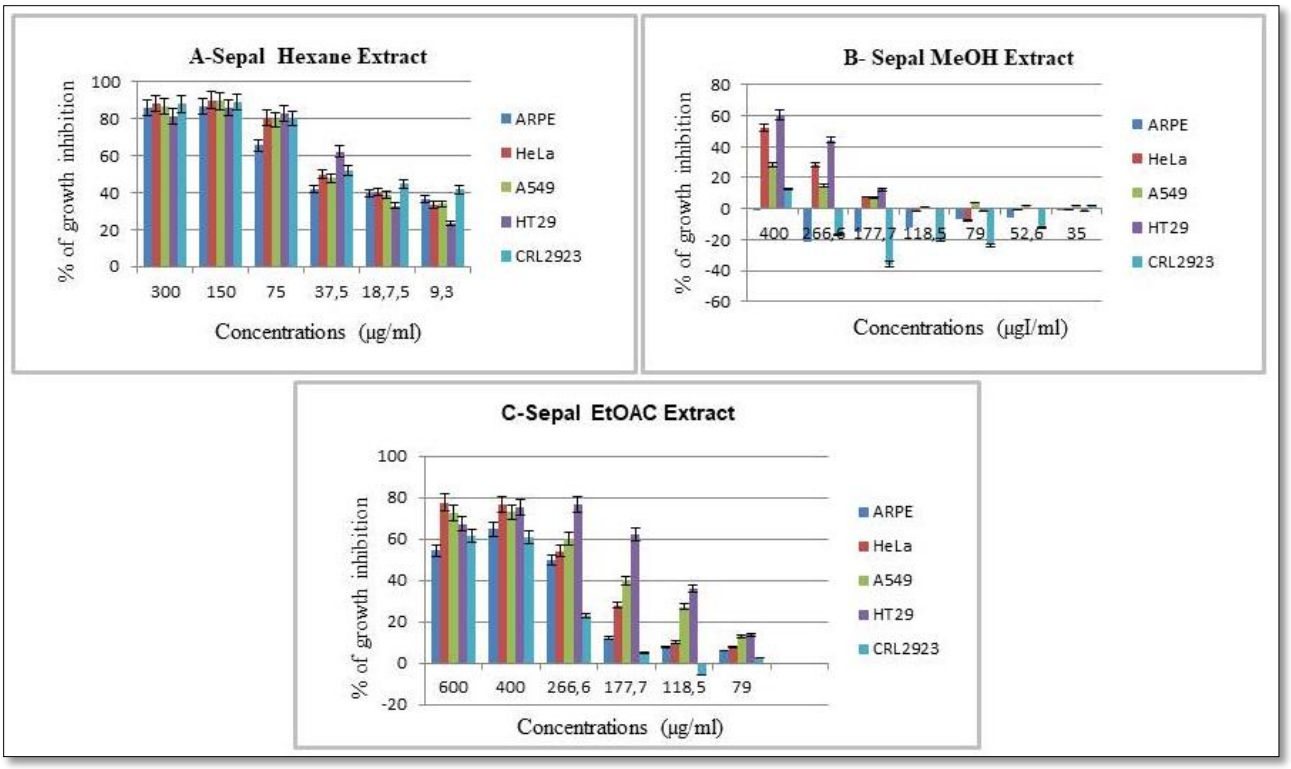

Figure 1. Anti-proliferative effects of sepal extracts from *R. luteum*.
Dose dependent inhibitory effects of Rhododendron luteum sepal extracts on cell viability of diploid and cancer cell lines determined using the MTT assay as described in materials and methods. The cells were exposed to Sepal hexane (A), MeOH (B) and EtOAc (C) extracts or the equivalent amount of DMSO for 48 h. The results are presented as mean of at least three independent experiments with two replicates at each point.

Cytotoxicity of petal extracts (Hexane, EtOAc): The hexane and EtOAc extracts from *R. luteum* petals were studied for their anti-proliferative effects on the growth of the non-transformed and the transformed cell lines. All the cell lines were treated by various concentrations of the hexane extract ranging from (500 to 15.6 µg/ml), EtOAc extract ranging from (800 to 105.3 µg ml) or consisting of the corresponding DMSO (max. 0.4%) in *in vitro* for 48 h conditions.

As shown in **Figure 2A**, exposure of non-transformed and transformed cell lines to decreasing concentrations of petal hexane extract caused dose-dependent inhibition of cell growth. On the other hand, the anti-proliferative effects of the extract on the HeLa cells were more evident at the 500 µg/ml and 250 µg/ml concentrations, causing growth inhibition by 58.7% and 46.4%, respectively, in comparison to the other cancer cell lines that were used in the study. Higher cytotoxic values indicated that the HeLa (cervical cancer) cells were the most sensitive cancer cells to the petal hexane extract as in comparison to the other three cancer cell lines (A549, HT-29, CRL2923). Nevertheless, the results revealed that there was no statistically significant difference between ARPE-19 and any other cancer cell line.

Treatment of the non-transformed and the transformed cell lines with various concentrations of the EtOAc extract from the petals of *R. luteum* for 48 h in *in vitro* conditions resulted in a concentration-dependent decline in cell viability **(Figure 2B)**. The IC_{50} values were calculated as 800 µg/ml, 644.4 µg/ml, 622.1 µg/ml, 459.3 µg/ml and 800 µg/ml for ARPE, HeLa, A549, HT-29 and CRL2923, respectively. Additionally, we found that the EtOAc extract from the petals at the concentrations of 533.5 µg/ml and 355.5 µg/ml induced the highest cytotoxic effects on HT-29 and showed growth inhibition by 65.67% and 41.99%, respectively **(Figure 2B)**. The same concentrations of the extract decreased cell viability by 20.2% and 6.29% in ARPE-19 cells, respectively. In terms of the cytotoxicity, the statistical analysis revealed that the difference between the ARPE-19 and HT-29 cells was significant ($p<0.05$). However, there was no significant difference between ARPE-19 and the other transformed cell lines. To summarize, the findings provided evidence that the EtOAc extract from *R. luteum* petals might have some compound/compounds that may induce specific anti-proliferative effects on HT-29 colorectal cancer cell line.

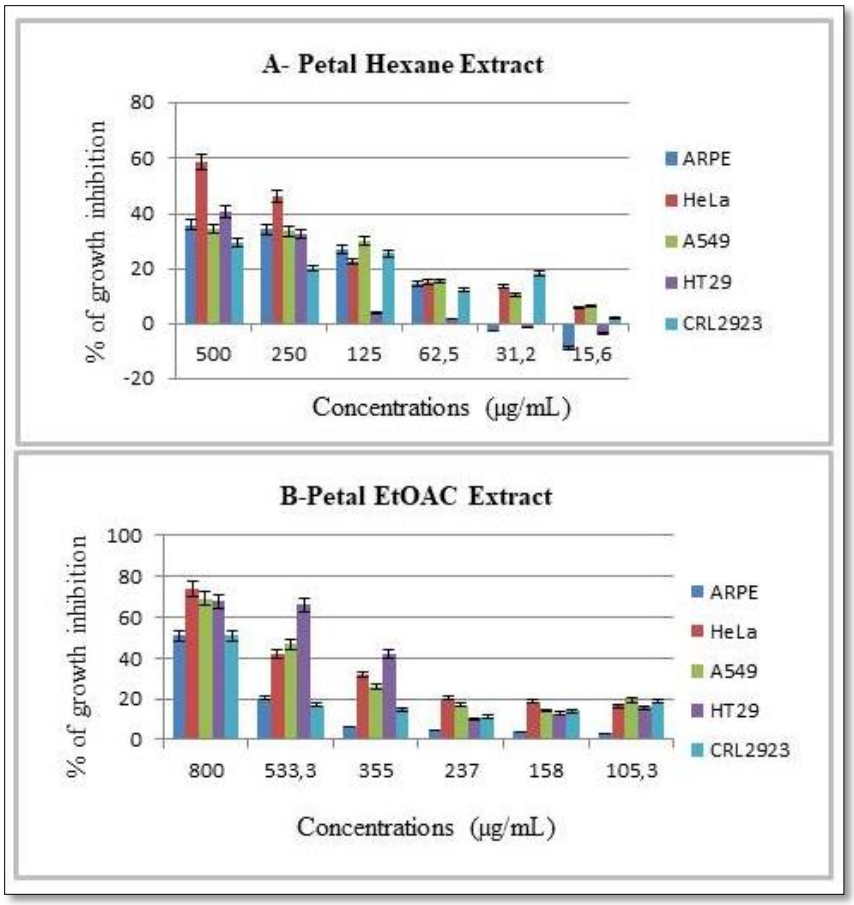

Figure 2. Anti-proliferative effects of petal extracts from *R. luteum.*

Dose dependent inhibitory effects of Rhododendron luteum petal extracts on cell viability of diploid and cancer cell lines determined using the MTT assay as described in materials and methods. The cells were exposed to petal hexane (A) and EtOAc (B) extracts or the equivalent amount of DMSO for 48 h. The results are presented as mean of at least three independent experiments with two replicates at each point.

Cytotoxicity of bud extracts (Hexane, MeOH, EtOAc): The non-transformed and the transformed cell lines were treated with various concentrations of the hexane, MeOH and EtOAc extracts obtained from the buds of *R. luteum*. As it can be seen in **Figure 3A**, the exposure of the cell lines to decreasing concentrations (500 µg/ml to -15.62 µg/ml) of bud hexane extract was found to be not effective on the ARPE-19, HT-29 and A549 cell lines. On the other hand, the extract induced mild anti-proliferative effects in both HeLa and CRL2923 cancer cells. Since both cell lines originated from human uterus tissues, the data may suggest that the extract may have selective anticancer activity against uterus tissues originated cancer cells. It is also interesting to note that the hexane extract of buds significantly enhanced the proliferation of non-transformed ARPE-19 cells especially at higher concentrations (500 µg/ml to -125 µg/ml). In the light of this information, it brings in mind that this extract has some components that might have a mitogen activity on non-transformed cells.

Figure 3B shows the anticancer activity of the MeOH extract of buds on normal human cell line (ARPE-19) and four cancer cell lines (ARPE, HeLa, A549, HT-29, CRL2923). The effect of the extract on human cell lines was tested at concentrations ranging from 400 to 52.6 µg/ml or DMSO (control) alone for 48 h in *in vitro* conditions. The results showed that the MeOH extract of *R. luteum* buds exhibited a dose-dependent cytotoxic effect for all the cancer cell lines that were evaluated. The extract at the concentrations of 400 µg/mL exhibited moderate cytotoxic effects on ARPE-19 cells by killing only 21.3% of the cells. However the same concentration of the extract showed higher cytotoxic activity against the cancer cell lines of HeLa, A549 and HT-29 with growth inhibition by 42.2% 47.3% and 60.2%, respectively **(Figure 3B)**. In addition to this, the difference between the ARPE-19 and transformed cell lines (A549, HT-29, HeLa) was found to be statistically significant ($p<0.05$).

Finally, we evaluated anti-proliferative effects of the EtOAc extract of *R. luteum* buds. It was determined that, at the

concentrations of 400 µg/ml, the extract was highly cytotoxic and led to ≥ 70% cell death in all the cell lines that were evaluated. However, as the concentrations were reduced gradually to 177.7 and 118.5 µg/ml, there were statistically significant differences the between non-transformed ARPE-19 and transformed cell lines (A549, HT-29, HeLa). As it can be seen in **Figure 3C**, the treatment of the cells with the 177.7 µg/ml concentration of the extract induced 74.28% cell death in the HT-29 cell while produced only 6.45% growth inhibition in normal ARPE-19 cell line. The IC_{50} values were found to be 366.6, 231.1, 222.1, 159.94 and 333.5 µg/ml for ARPE-19, HeLa, A549, HT-29 and CRL2923, respectively. Additionally, the results revealed that the extract was more potent and more selective against the HT-29 colon cancer cells showing the highest SI (selectivity index) as 2.29.

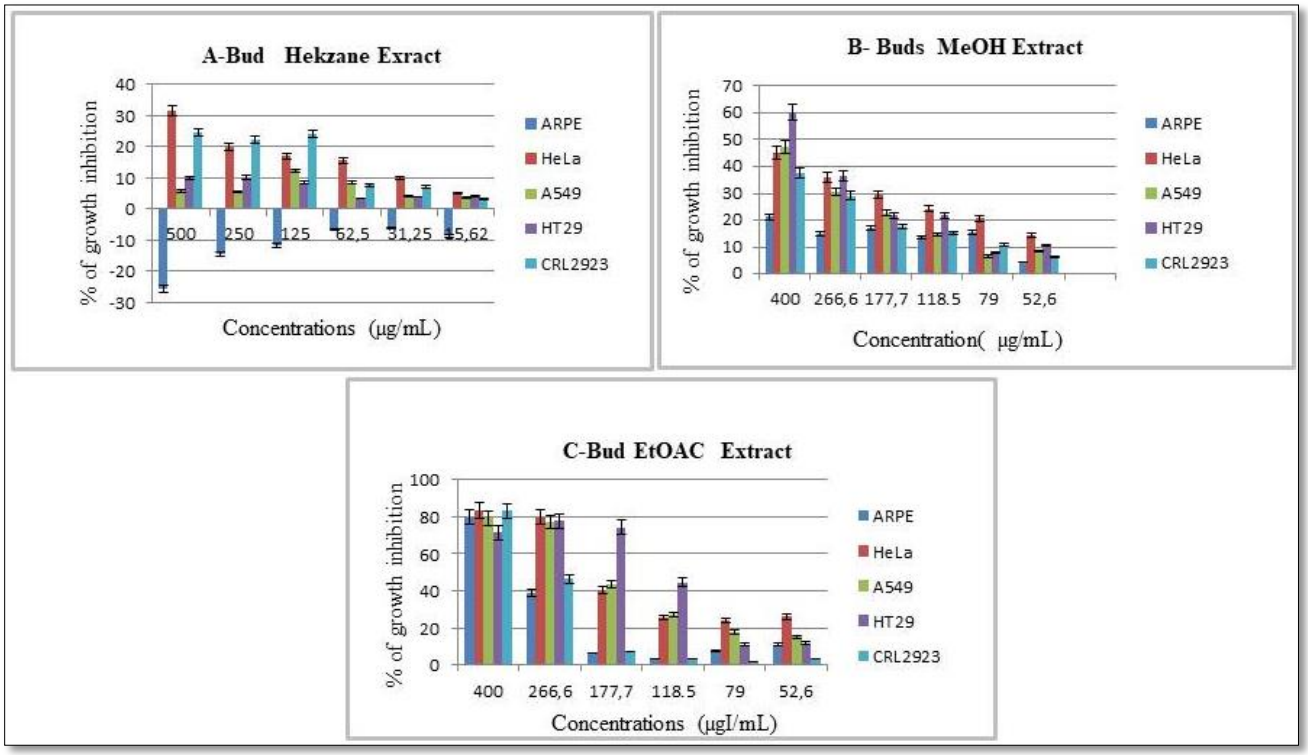

Figure 3. Anti-proliferative effects of bud extracts from *R. luteum.*

Dose dependent inhibitory effects of Rhododendron luteum bud extracts on cell viability of diploid and cancer cell lines determined using the MTT assay as described in materials and methods. The cells were exposed to bud hexane (A), MeOH (B) and EtOAc (C) extracts or the equivalent amount of DMSO for 48 h. The results are presented as mean of at least three independent experiments with two replicates at each point.

Morphological changes

To observe the morphological changes, the cells (ARPE-19, A549, HT-29, HeLa) were treated with 300 µg/ml of *R. luteum* EtOAc extract from the sepals, Taxol, media or DMSO alone. After 24 h of incubation, the cells were examined under an inverted microscope. As shown in **Figure 4A**, no morphological changes were observed in the cells treated with negative controls (DMSO or media). However, Taxol (5 nM) was used as a positive control, which caused rounding and detachment of the transformed cells **(Figure 4A)**. Meanwhile, exposure of the diploid ARPE-19 cells to the EtOAc extract (300 µg/ml) for 24 h caused no apparent morphological alterations in these cells. However, similar to Taxol, the EtOAc extract was found to cause cellular death in the HT-29 and HeLa cell lines by breaking the cells from the surface **(Figure 4A)**. Additionally, among the transformed cell lines, the least morphologically affected one was the A549 cell line.

Apoptotic activity analysis

In recent years, there has been considerable interest in treatment-induced apoptosis. Formation of DNA fragmentation is one of the characteristic features of apoptosis [26]. Therefore, the controls and EtOAc induced apoptosis were performed by the DNA fragmentation assay using agarose gel electrophoresis and Hoechst (H33342) staining.

Hoechst staining and electrophroretic analysis of DNA showed that exposure of the cells to *R. luteum* EtOAc extract (400 µg/ml) for 48 h did not result in a characteristic chromatin condensation or DNA laddering pattern **(Figures 4B and 4C)**. Since these two are the biochemical hallmarks

of apoptosis, these data may suggest that the EtOAc extract might promote different signals to mediate cancer cell death. Over the past few years the role of autophagy in cancer has been reported in various studies [27-29]. Others showed that some anticancer agents [30] or some plant extracts [31,32] can promote necroptosis in cancer cells.

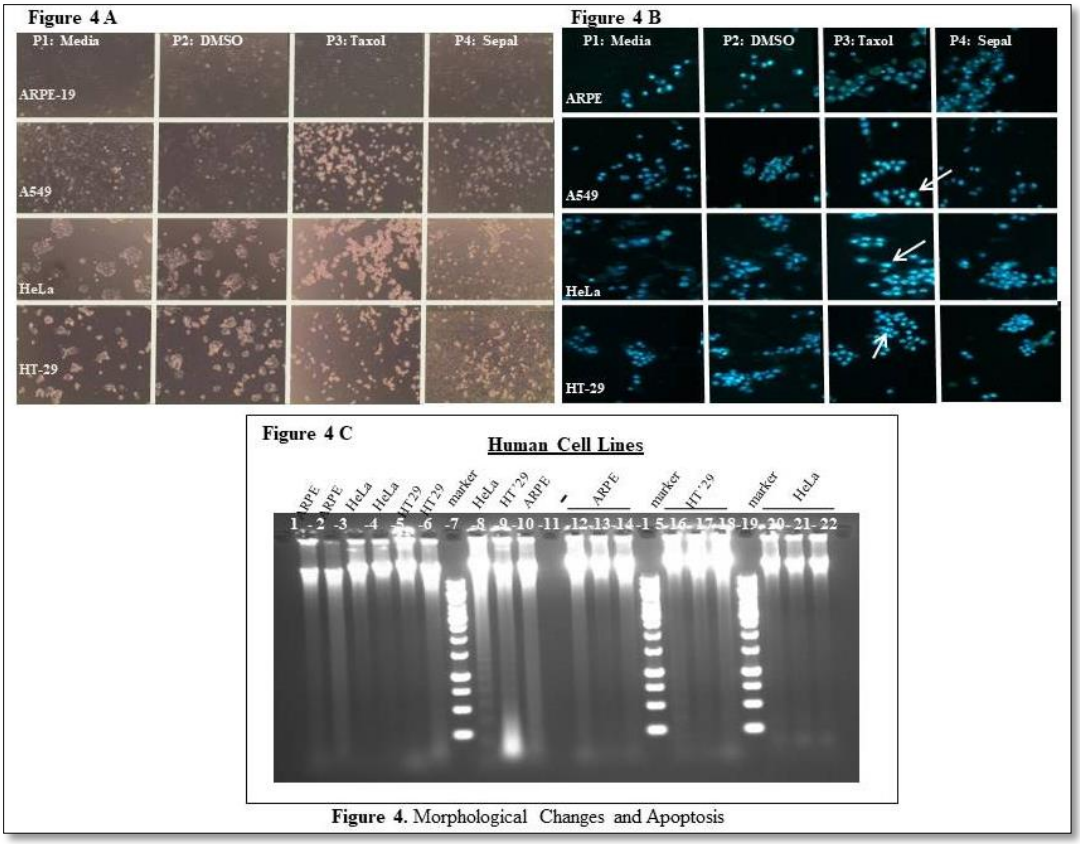

Figure 4. Morphological changes and apoptosis.

***A**) Effects of Rhododendron luteum sepal EtOAc extract (300 µg/ml), Taxol (5 nM), DMSO or medium on the morphology of the diploid and cancer cell lines. Morphological changes of the cells were examined under an Olympus inverted microscope using 20x objective lens. **(B)** Cell apoptosis observed using fluorescence microscope (20x). For detection of apoptosis, ARPE (diploid) and cancer cell lines (HeLa, A549, HT-29, CRL2923) were incubated with Rhododendron luteum sepal EtOAc extract (300 µg/mL), Taxol (5 nM), medium or the equivalent amount of DMSO for 48 h. The apoptotic cells were observed with fluorescence microscope after Hoechst 33342 staining. Arrows show the apoptotic cells (condensed/ fragmented nuclei). **(C)** Effects of Rhododendron luteum sepal EtOAc extract (400 µg/mL, Taxol, medium or the equivalent amount of DMSO on DNA fragmentation in diploid and cancer cell lines. Lane: 1, 3, 5 (medium alone), Lane: 3, 4, 6 (DMSO), Lane: 8-10 (Taxol), Lane: 7, 9, 15 marker (1 kb-100 bp), For EtOAc extract: Lane: 12-14, ARPE-19 cells, Lane: 16-18 HT-29 cells, Lane: 20-22 HeLa cells.*

DISCUSSION

Thousands of years ago, human beings found the power of plants for treatment [7-33]. In the past few years, the use of some remedies has attracted a great deal of attention for alternative cancer therapies and some of them have been reported to inhibit the growth of human cancer cell lines [34-37].

Rhododendron is one of the largest genera of plants [17] and *R. luteum* is a well-known poisonous plant that widely grows and is distributed in the Black Sea region of Turkey [38]. Very limited information is available regarding the biological activity of *R. luteum.* However, among herbal and plant-base medicines, Rhododendrons have gained prominence in recent years for their bioactive compounds and their anti-carcinogenic potential [14]. Previously, it was reported that the some species of the Rhododendron genus contain high levels of essential oils [39] and flavonoid [40,41] compounds in their chemical composition. It was also, documented that both the essential oils [39] and flavonoids may display anti-carcinogenic activity against different cancer cell lines [42,43]. Additionally, another study reported that flavonoids may increase susceptibility of cancer cells to chemotherapy [44]. Therefore, Rhododendron species may have prominence due to their anti-carcinogenic potential.

The goal of this study was to understand whether or not *R. luteum* is a medicinal herbal plant. Therefore, extracts were prepared using *R. luteum* flower parts (sepals, petals) and buds in different solvents including MeOH, EtOAc and hexane. All extracts were tested for their anticancer activity using non-transformed (ARPE-19) and transformed (HeLa, A549, HT-29 and CRL2923 cell lines. The cytotoxic activities of the extracts were determined by MTT assay and morphological alterations.

First of all, MeOH extract of the sepals was found to have no anti-proliferative effect on non-transformed ARPE-19 cells, whereas the extract was more prominent in the HeLa and HT-29 cells in comparison to A549 and CRL-2923 **(Figure 1A)**. MeOH extract from the buds caused moderate cytotoxicity on ARPE-19 cells, while extract showed a dose-dependent cytotoxic effect on all the cancer cells **(Figure 3B)**. These findings suggest that MeOH extract of *R. luteum* exhibit cytotoxic effect against broader range of the cancer cell lines. Therefore, the extract might have antitumor potential against cancer that originated from the different tissues.

Next, we tested the anti-proliferative effects of hexane extracts that were obtained from *R. luteum*. Even though, administration of the sepal hexane extract on non-transformed and transformed cells caused dose-dependent death of the cells **(Figure 1A)**, the petal and bud hexane extracts gave rise to consistently higher cytotoxic effect on HeLa cervical cancer cells at high concentrations **(Figures 2A and 3A)**. Our results here indicated a surprising finding that the hexanic extract from the buds had no harming effects on non-transformed ARPE-19 cells, while it actually significantly enhanced the proliferation of the ARPE-19 cells especially at high concentrations **(Figure 3A)**. One possible reason for the finding might be that, despite its anti-proliferative activity on some cancer cell lines, the extract may play a role as a mitogen on non-dividing or slow-dividing cells. This needs to be further studied to be verify the possible mitogenic activity of the bioactive compounds that might present in the hexane extract.

This study also focused on the anti-proliferative effects of the EtOAc extracts obtained from different parts of the flowers (sepal, petal) and the buds of *R. luteum.* As shown in **Figures 1C, 2C and 3C** at high concentrations all the extracts were shown to possess high cytotoxicity against all the cells lines that were used in the study. However, as the concentrations of the extracts were gradually reduced, the results revealed that extracts from the sepals, buds or petals were more potent and more selective against HT-29 colon cancer cells showing higher SI (selectivity index) values as 2.0, 2.3 and 1.7, respectively, in comparison other cancer cell lines. This finding might be important since it was reported that, if a compound has a SI value above 3 that compound may potentially be used to develop anticancer agents [25]. Given that the samples assayed in the study were not purified compounds, the extracts may have so many compounds with different biological activities. These results provide a possible lead towards further studies to find out the chemical composition of the extracts including the individual compounds which may mediate selective activity against HT-29 colon cancer cells.

Although there is a limited number of studies regarding the anti-proliferative activities of *R. luteum* in *in vitro* conditions, our results reported above were consistent with those of Selim Demir et al. [45] which indicated that extracts from *R. luteum* had specific cytotoxicity against WiDr colon cancer cell line. In a study conducted in 2011, three different components (Ferruginenes A, B, C) obtained from the leaves of *R. ferrugineum* were tested for their cytotoxic effects on the transformed (HT-60, HeLa, S3, MCF-7) and non-transformed (HEK-293) cell lines. As a result they reported that Ferruginenes (A, B, C) were the most effective against HL-60 human cell line [46]. In another study, the researchers showed that essential oils obtained from the leaves and flowers of *R. antropogoni* induced more cytotoxic activity on Cervix A-143 cells in comparison to other human adenocarcinoma cell lines (Ovarian-2008, LoLo-colon) [39]. In related studies, Way et al. [47,48] showed that components that were obtained from *R. formosanum* leaves enhanced cell death in non-small-cell lung carcinoma cells. Our results agreed with the reports above on that extracts from *Rhododendron* spp. may have different anti-proliferative properties in different cancer cell lines.

CONCLUSION

In conclusion, identifying differential cytotoxicity is important for developing potential anticancer agents. The overall findings of this study indicated that the hexane extracts derived from *R. luteum* showed selective anti-proliferative activity more against the HeLa cervical cancer cells, whereas the MeOH extracts from *R. luteum* affected a broader range of the cancer cell lines similarly. The encouraging result obtained from this study was that HT-29 cells were particularly susceptible to EtOAc extracts of *R. luteum*, which may finally lead us to think that these extracts may have some specific compounds that are responsible for the anti-proliferative activity on these cells. This suggests that further studies are needed to better understand the mechanisms behind these inhibitory effects that lead to cell death in HT-29 cell line. Therefore, we will pursue isolation and characterization of the specific compounds responsible for this biological activity and investigate its detailed molecular mechanism.

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