Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Antiproliferative effects of hazelnut cell culture extract on the different cancer cell lines

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Article Info

Article history:

Received: June 12, 2024
Accepted: September 30, 2024
Published: November 30, 2024

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Abstract

The increasing incidence of cancer has necessitated the discovery of novel anticancer compound sources. The presence of taxanes in hazelnut cell cultures has promoted new promising pharmacotherapeutic applications. The antiproliferative properties of hazelnut (*Corylus avellana* cv. 'Kalınkara') cell culture extracts against different human cancer cell lines (HeLa, MCF-7, MDA-MB-231, A549) with Beas-2B as control were evaluated. The cytotoxicity of *C. avellana* culture extract (5 µM, 10 µM, and 20 µM) on all cell lines was evaluated with xCELLigence Real Time Cell Analysis System. Mitotic activity (450-655 nm), BrdU activity (450-550 nm) and caspase 3,7 activity (490-520 nm) were analyzed with a spectrophotometer through 24, 48, and 72 hours. Based on the values obtained from the xCELLigence Cell Analysis System, a 10 µM concentration of the culture extract was assigned as the IC_{50} dose. Culture extracts at 10 µM enhanced the reduction in the proliferation of all cancer cells assayed. The highest decrease in mitotic (59.32%) and BrdU (53.77%) activity was observed in A549 lung cancer cells. However, caspase 3,7 activity (35.08%) was the highest in aggressive MDA-MB-231 breast cancer cells. The culture extracts decreased the viability of A549 cells to a greater extent than that of MCF-7 and MDA-MB-231 breast, and HeLa cervical cancer cells. *C. avellana* cv. 'Kalınkara' cell culture extracts have potential use in the treatment of lung and, to a lesser extent, breast and cervical cancers.

Keywords: Anti-cancer, Cancer cell lines, Cell culture extract, Cell viability, *Corylus avellana*, Taxanes

1. Introduction

Cancer is a crucial public health issue worldwide that affects both industrialized and developing nations [1]. Owing to the notable nature of the disease, a constant struggle is sought with low success in treatment [2]. Considering its current situation, it is necessary to investigate a drug type with reduced toxicity, fewer side effects, and more effective for cancer treatment or adjuvant therapy [3]. The result of the wide range of searching for the most effective products to cure cancer was the discovery lifesaving compound paclitaxel. Paclitaxel was extracted from the Pacific yew tree (*Taxus baccata*) and later became the cancer drug Taxol® [4]. Since the discovery of paclitaxel's antitumoral activity, Taxol® approved as a chemotherapeutic drug for the treatment of breast, ovarian, stomach, prostate, lung, bladder, Kaposi sarcoma and esophageal cancers [5].

Some plant-derived substances such as curcumin, resveratrol, berberine, vinca alkaloids and taxanes (Taxol® and Docetaxel®) are often used to treat cancer patients [6]. However, the most frequently prescribed anticancer medications by doctors are taxanes, which are now understood to restrict and impede cell development, differentiation, and proliferation in most recognized cancer cell lines. Everyone, including patients in the oncological profession, greatly appreciates their methods of reducing cell development, whether in experimental or clinical studies [7, 8].

A group of anticancer medications known as "taxanes" work by attaching to microtubules and tubulins, which are essential for cell division. Microtubule dynamics are stabilized by the binding of taxanes to β-tubulin, which encourages microtubule construction while concurrently inhibiting disassembly. Microtubule dynamics are suppressed, which blocks mitosis and causes cell death [9]. Although cell division arrest at the G2/M stage, which initiates the signalling pathway that causes apoptosis, is the major mechanism of action, there appear to be additional processes that work simultaneously to make these medications effective. Additionally, these medications are frequently used with other medications that work in different ways due to their distinct mode of action [10].

The discovery of taxanes in hazelnut (*Corylus avellana* L.) has provided novel insights into their chemotherapeutic activities [11]. *C. avellana* has many secondary metabolites with pharmacologic activity including phenolic acids, flavonoids, tannins, proanthocyanidins, diarylheptanoids, lignans, and taxanes. Gallego *et al.* (2017) reported

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Doi: http://dx.doi.org/10.14715/cmb/2024.70.11.13

the viability-reducing activity of hazelnut tree extracts in different cancer lines [12]. In addition, plant cell culture systems offer an eco-friendly and sustainable platform for enhanced taxane production. Anticancer activities of *C. avellana* cell suspension cultures were successfully assessed against breast, cervical and lung cancer cells [13, 14, 15]. However, anti-proliferative activity of Turkish hazelnuts has not been determined to date.

Turkey, the largest hazelnut producer worldwide, has 18 hazelnut cultivars [16]. Kutlutürk *et al.* (2024) found that seven Turkish hazelnuts accumulated prominent taxanes including 10-deacetylbaccatin III, baccatin III, cephalomannine and paclitaxel in their various tissues [17]. Cell suspension cultures of Kalınkara, best adapted to the tissue culture systems among the seven cultivars were established to evaluate their taxane production capacity based on the effects of elicitors such as methyl jasmonate, coronatine, phenylalanine and methyl-β-cyclodextrin [18, 19]. This study aimed to investigate taxane-dependent antiproliferative effects of the methanolic extracts obtained from hazelnut (*Corylus avellana* cv. 'Kalınkara') cell suspension cultures against lung (A549), breast (MCF-7, MDA-MB-231) and cervical (HeLa) cancer cell lines with non-tumorigenic epithelial cell line Beas-2B as a control. IC_{50} dose was found by using xCELLigence Real-Time Cell Analysis System. With this study mitotic, BrdU, and caspase 3,7 activities of the *C. avellana* cv. 'Kalınkara' cell culture extract against different cancer cells were assessed to determine its cytotoxic activities the first time. The results might offer an invaluable resource for the pharmaceutical industry for large-scale taxane production.

2. Material and Methods

2.1. Plant tissue culture and taxane extraction

Corylus avellana cv. 'Kalınkara' hazelnuts were kindly donated by Prof. Dr. Umit Serdar from Ordu province (40°59'27.9"N 37°35'01.6" E), Turkey in 2021. Callus cultures were established as described by Goktepe-Atilgan *et al.* (2023) [19]. Cell suspension cultures were derived from embryogenic calli. The establishment of the suspensed cultures was performed as described by Dogan (2023) [18]. Extraction procedures of taxanes from the cultures were followed Gallego *et al.* (2015) [21].

2.2. Cancer cell culture and reagents

RPMI-1640 was used for breast (MCF-7), lung (A549), and Beas-2B (non-tumorigenic) lines, while high-glucose Dulbecco's Modified Eagle's Medium (Gibco Co, USA) was used for aggressive triple-negative breast (MDA-MB-231) and cervical (HeLa) lines. Each medium contains 10% (v/v) fetal bovine serum (Gibco Co, USA) and 100 µg/ml streptomycin (Streptomycin sulphate, I. E. Ulugay), 100 IU/ml penicilin (Pronapen, Pfizer). All cells were grown at 37°C under conditions of 5% CO_2 and a humid atmosphere. All cell lines used in the experiments were provided by American Type Culture (ATTC Manassas, VA, USA).

2.3. Real-time cell analysis

The cytotoxicity of *C. avellana* culture extract on all cell lines was determined with xCELLigence Real Time Cell Analysis System (Roche, USA). E-plate with 16 wells was used in xCELLigence DP. Cells were planted in each well as 10 x10^3 for MCF-7, 5 x10^3 cells for MDA-MB-231, 8 x10^3 cells for HeLa, 8 x10^3 cells for A549, and 5 x10^3cells for Beas-2B. The device was set to perform cell analysis every 15 minutes for 100 hours. Different concentrations (5 µM, 10 µM and 20 µM) of *C. avellana* culture extract were added after approximately 21 hours of incubation, and time-dependent graphs were obtained by continuing to take measurements.

2.4. Mitotic activity analysis

Mitotic Assay Kit was used for the determination of the mitotic activity of cells applied to *C. avellana* culture extracts (10 µM) through 24, 48, and 72 hours according to the manufacturer's protocol (Active Motif, Cat No: 18021). The cells were analyzed with a spectrophotometer (Flx-800, Bio-Tek Instruments Inc.) at 450-655 nm wavelength.

2.5. BrdU activity analysis

BrdU Cell Proliferation Kit was utilized for the determination of DNA synthesis. The experiment was carried out in accordance with Millipore protocol (Cat No:2750). The cells which applied to *C. avellana* culture extracts (10 µM) through 24, 48, and 72 hours were analyzed with a spectrophotometer (Flx-800, Bio-Tek Instruments Inc.) at 450-550 nm wavelength.

2.6. Caspase 3,7 activity analysis

Caspase 3,7 activity detection was carried out for determination of apoptosis using CaspaTag Caspase 3,7 In Situ Assay Kit Fluorescein™ according to manufacturer's protocol (Millipore, Cat No: apt423). *C. avellana* culture extracts (10 µM) were applied to per cell lines for 24, 48, and 72 hours. Using the Fluorescent Plate Reader (Flx-800, Bio-Tek Instruments Inc.), measurements were made at 490 nm excitation wavelength and 520 nm emission wavelength.

2.7. Statistical analysis

The data of the experimental groups was compared with a one-way ANOVA test. Statistical analysis has been done using GraphPad Prism software (California, USA). A $p < 0.05$ significance level was accepted in the tests.

3. Results

3.1. Cell index

Cell index can be employed to measure the state of the cell based on the observed cell-electrode impedance. Cell index values obtained from xCELLigence DP showed that the application of *C. avellana* culture extracts on HeLa, MCF-7, MDA-MB-231, and A549 cells had significant anti-proliferative effects (Figure 1-5.). However, the application of the culture extract on Beas-2B cells did not show any effect as in cancer cell lines. When the xCELLigence curves of HeLa cells were compared, it was thought that 5 µM and 10 µM concentrations of the culture extracts had cytostatic effects, while its concentration (20 µM) stimulated DNA damage. It was thought that the MCF-7 cell line with a 5 µM concentration of the culture extract promoted DNA damage, while its concentrations (10 µM and 20 µM) had a cytostatic effect. All concentrations of the culture extracts caused DNA damage in the MDA-MB-231 cells. Graphical curves of the A549 cell line showed that 5 µM concentration of the culture extract did not significantly decrease, but its concentration (10 µM) had anti-mitotic

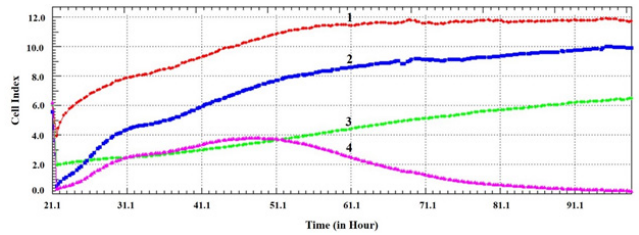

Fig. 1. Cell index values of HeLa cells treated with 5 µM, 10 µM and 20 µM concentrations of *C. avellana* culture extract obtained from xCELLigence Real-Time Cell Analysis System (1: Control, 2: 5 µM, 3: 10 µM, 4: 20 µM).

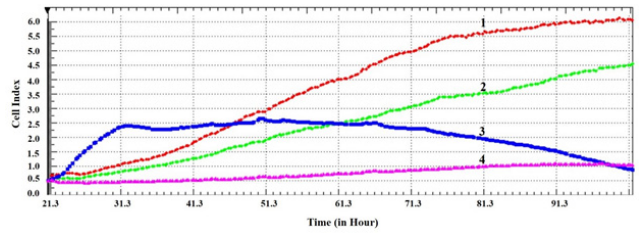

Fig. 2. Cell index values of MCF-7 cells treated with 5 µM, 10 µM and 20 µM concentrations of *C. avellana* culture extract obtained from xCELLigence Real-Time Cell Analysis System (1: Control, 2: 5 µM, 3: 10 µM, 4: 20 µM).

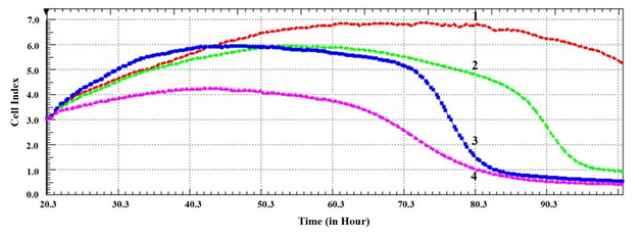

Fig. 3. Cell index values of MDA-MB-231 cells treated with 5 µM, 10 µM and 20 µM concentrations of *C. avellana* culture extract obtained from xCELLigence Real-Time Cell Analysis System (1: Control, 2: 5 µM, 3: 10 µM, 4: 20 µM).

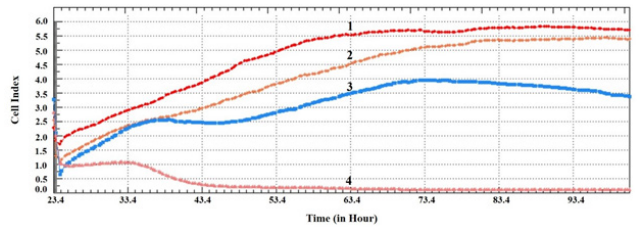

Fig. 4. Cell index values of A549 cells treated with 5 µM, 10 µM and 20 µM concentrations of *C. avellana* culture extract obtained from xCELLigence Real-Time Cell Analysis System (1: Control, 2: 5 µM, 3: 10 µM, 4: 20 µM).

activity and its concentration (20 µM) had cytoskeletal effects. Graphs of Beas-2B cells did not show a significant decrease at any concentration compared to cancer cells. All concentrations showed cytostatic effects.

3.2. Mitotic activity analysis

The assessment of mitotic activity is a key factor in the evaluation of the antiproliferative effects of various cancers. The *C. avellana* culture extract (10 µM) showed time-dependent anti-mitotic activity in all the cancer cell lines (Figure 6.). Mitotic activity decreased from 24 to 72 h in all cancer cell lines. The highest reduction (59.32%) of this activity was observed in A549 lung cancer cells at 72 h after adding 10 µM culture extract, and the lowest reduction (30.26%) was observed in non-tumorigenic epithelial cells (Beas-2B) as a control. It similarly decreased mitotic activity in MCF-7 (56.54%) and MDA-MB-231 (50.47%) breast cells and HeLa (50.49%) cervical cancer cells at 72 h.

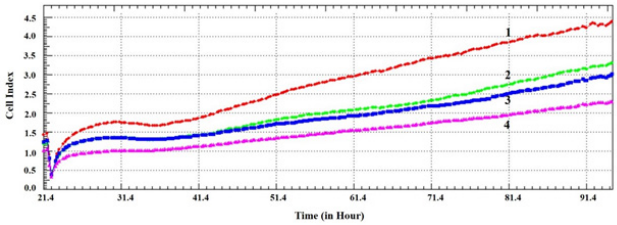

Fig. 5. Cell index values of Beas-2B cells treated with 5 µM, 10 µM and 20 µM concentrations of *C. avellana* culture extract obtained from xCELLigence Real-Time Cell Analysis System (1: Control, 2: 5 µM, 3: 10 µM, 4: 20 µM).

3.3. BrdU activity analysis

BrdU is a synthetic thymidine analog that is incorporated into the DNA during replication. BrdU activity analysis was performed for cell proliferation. The results showed that 10 µM *C. avellana* culture extract affected time-dependent BrdU activity in all cancer cell lines (Figure 7.). BrdU activity decreased from 24 to 72 h in all cancer cell lines. The highest reduction (53.77%) of this activity was observed in A549 lung cancer cells at 72 h after adding 10 µM culture extract, while the lowest reduction (30.99%) was observed in non-tumorigenic epithelial cells (Beas-2B) as a control. It decreased BrdU activity in MDA-MB-231 (52.09%) breast cancer cells compared to

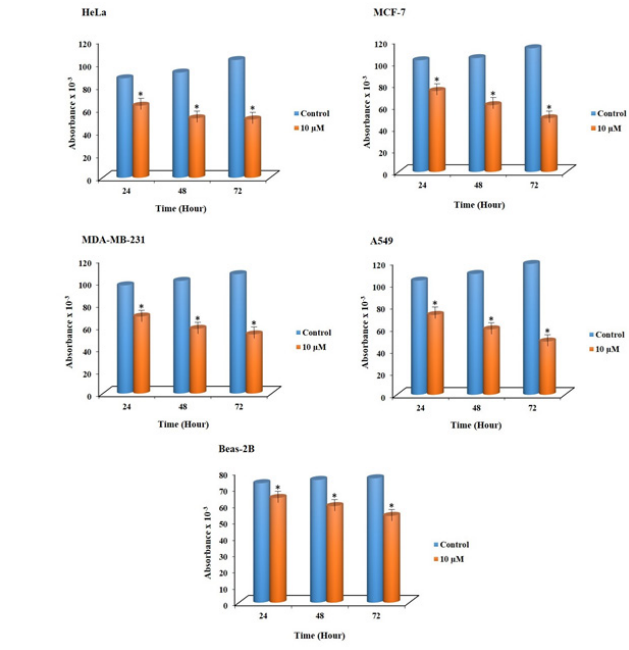

Fig. 6. Absorbance values belong to mitotic activity of HeLa, MCF-7, MDA-MB-231, A549 and Beas-2B cells treated with 10 µM *C. avellana* culture extract for 24-72 hours ($p<0.05$ (*) significance level was accepted in the tests).

MCF-7 (41.58%) and HeLa (35.62%) cancer cells at 72 h.

3.4. Caspase 3,7 activity analysis

A class of cysteine proteases known as caspases targets and hydrolyzes particular target proteins. The activation of caspases is thought to be a crucial indicator of planned cell death since it guarantees that cellular constituents are broken down in a regulated way. The results showed that 10 µM *C. avellana* culture extract affected time-dependent caspase 3,7 activity in all cancer lines (Figure 8.). Caspase 3,7 activity increased from 24 to 72 h in all cancer cell lines. While the highest reduction (35.08% and 32.55% respectively) of caspase 3,7 activity was recorded in MDA-MB-231 breast and A549 lung cancer cells at 72 h after adding 10 µM culture extract, the lowest reduction (12.86%) was observed in non-tumorigenic epithelial cells (Beas-2B) as a control. It decreased caspase 3,7 activity in MCF-7 (26.94%) breast cancer cells compared to HeLa (14.04%) cervical cancer cells at 72 h.

4. Discussion

Taxane and taxane derivative compounds are still used effectively in cancer treatment, and an increasing number of taxane derivatives are required every day. Alternative methods for the production of taxane-derived substances are being investigated for reasons such as the low yield of production in Taxus species and the fact that it takes a long time for trees to reach sufficient maturity for extraction. In the pharmaceutical production industry, cell culture studies are carried out on a large scale in the laboratory using biotechnological production methods. Working with cell cultures under laboratory conditions has important advantages, such as optimizing culture conditions, high productivity, cost-effectiveness, and suitability for high biomass production [22]. In this research, the anti-cancer effects of taxanes, which were extracted from cell suspension cultures of the Kalınkara cultivar of Turkish hazelnut

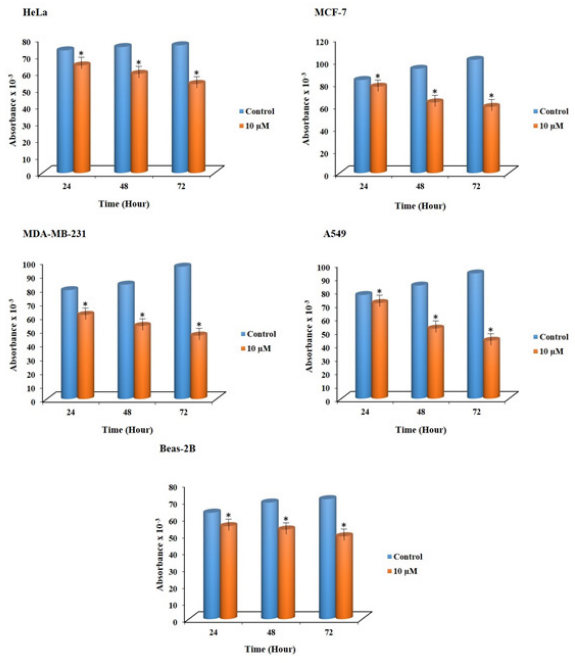

Fig. 7. Absorbance values belong to BrdU activity of HeLa, MCF-7, MDA-MB-231, A549 and Beas-2B cells treated with 10 µM *C. avellana* culture extract for 24-72 hours ($p<0.05$ (*) significance level was accepted in the tests).

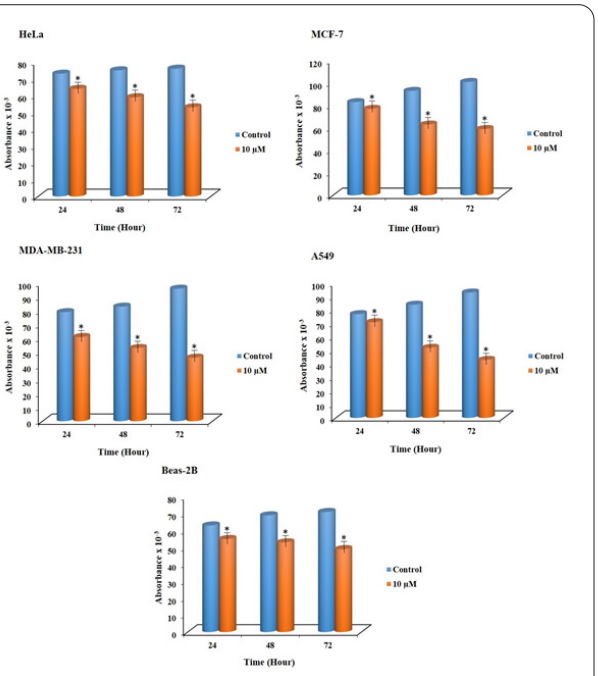

Fig. 8. Fluorescence values belong to caspase 3,7 activity of HeLa, MCF-7, MDA-MB-231, A549 and Beas-2B cells treated with 10 µM *C. avellana* culture extract for 24-72 hours ($p<0.05$ (*) significance level was accepted in the tests).

(*C. avellana* L.), previously shown to contain taxane [17, 18, 19], were determined using different cancer cell lines.

The most commonly used chemotherapeutic agents against many different forms of cancer belong to the taxane class of anti-cancer medications. It precisely arrests cells in the G2/M phase of the cell cycle and produces cytotoxicity in a manner that depends on time and concentration [23]. One of the most frequently used anti-cancer medications, paclitaxel, is effective against a variety of malignancies, including breast, ovarian, stomach, prostate, lung, bladder, Kaposi sarcoma esophageal, endometrial and cervical cancers [24]. Therefore, assessments of new paclitaxel sources are required to be crucial to meet worldwide demand rising rapidly to improve effective cancer treatments.

Hazelnut extracts decreased the viability of HeLa, HepG2, and MCF-7 lines [12]. In a study evaluating the anti-proliferative effects of *C. avellana* cell culture extracts, they were applied to the lung cancer cell line SK-Mes-1, which stopped the metaphase/anaphase transition with higher activity in cancer cells than yew tree extracts, the main taxane source. In addition, hazelnut cell extract was applied to the cancer cell line and showed more effective anti-proliferative activity than pure Taxol® [13]. Moreover, extracts of silver nanoparticle-treated *C. avellana* cells may stop the growth of malignant HeLa cells and lower their viability to 60% of the control [15]. These results confirmed the hypothesis that hazelnut cell culture extracts may contain other Taxol®-derived compounds that can strengthen their anti-cancer effects [14]. Similarly, taxane-dependent extract of Taxus spp. *in vitro* culture decreased cell viability in lung (A549), breast (MCF-7), colon (LS174T) and hepatoma (SMMC-7721) cancer lines [25]. In this study, 10 µM *C. avellana* cv. 'Kalınkara' culture extracts enhanced a higher reduction in the proliferation of all cancer cells assayed (HeLa, MCF-7, MDA-MB-231 and A549). The highest decrease in mitotic

(59.32%) and BrdU (53.77%) activities were determined in A549 lung cancer cells. However, caspase 3,7 activity (35.08%) was the most increased in MDA-MB-231 breast cells. The culture extracts decreased the viability of A549 cells to a greater extent than MCF-7, MDA-MB-231, and HeLa cells. Therefore, *C. avellana* cell culture extracts may serve as potential therapeutic agents for cancer treatment. Moreover, our results suggest the potential use of *C. avellana* cv. 'Kalınkara' cell suspension culture extracts in the treatments of lung cancers and, to a lesser degree, breast and cervical cancer. In conclusion, Kalınkara cell suspension culture extracts with high antiproliferative effects against different cancer cell lines might be considered extraordinary potential alternative sources for pharmaceutical and clinical use in the future.

5. Conclusion

It can be concluded that hazelnut cell suspension culture extracts showed antiproliferative effects on different cancer cell lines in low doses within the treatment period of 24-72 hours. As a result, it is anticipated to be a profitable focus of research for both its *in vivo* and *in vitro* anticancer properties, as well as a potential cancer therapy.

Conflict of interest

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Acknowledgements

The authors would like to thank Dr. Umit SERDAR for providing hazelnut seeds.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed Consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Sule Ari: Devised the project, the main conceptual ideas and supervision; İdil Cetin: Perform all cancer cell culture and activity analysis procedures; Ahmet Dogan: Perform all plant tissue culture and extraction procedures; Mehmet Rıfkı Topcul: Conceived the study and were in charge of overall direction and planning. All authors discussed the results and contributed to the final manuscript.

Funding

This work was supported by the Research Fund of Istanbul University under a Grant TSA-2020-34684.

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