

Evaluation of Cytotoxic, Genotoxic and Apoptotic Effects of Turkish Sea Buckthorn (*Hippophae rhamnoides* L.) Fruit Extracts in Human Glioblastoma and Melanoma Cell Lines

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Türk Yalancı İğdesi (*Hippophae rhamnoides* L.) Meyve Ekstrelerinin İnsan Glioblastoma ve Melanom Hücre Hatlarındaki Sitotoksik, Genotoksik ve Apoptotik Etkilerinin İncelenmesi

SUMMARY

Hippophae rhamnoides L., commonly known as sea buckthorn, has been traditionally used in Eastern Europe and Asia for its nutritional and medicinal properties. Sea buckthorn fruit rich in bioactive compounds with well-documented antioxidant properties; however, its anticancer potential remains insufficiently explored, particularly in aggressive cancer types. Glioblastoma and melanoma are classified as the most dangerous cancer types with high mortality rates. In this study, the cytotoxic, genotoxic, and apoptotic effects of 80% ethanol and n-hexane extracts of sea buckthorn fruits were investigated in human glioblastoma (U87) and melanoma (SK-MEL-30) cell lines. Cells were treated with extracts at concentrations ranging from 0.05 to 0.6 mg/mL. Cytotoxicity was assessed using the MTT assay, genotoxicity was evaluated using the Comet assay, and apoptosis was analyzed by Annexin V/PI flow cytometry. Both extracts exhibited significant cytotoxic activity against U87 and SK-MEL-30 cells, although the magnitude of the response varied according to concentration, exposure time, and extraction solvent. The n-hexane extract generally demonstrated stronger antiproliferative effects than the ethanolic extract, particularly after prolonged exposure. Comet assay analysis revealed significantly increased DNA damage in extract-treated cells compared with controls, indicating the induction of genotoxic stress. Flow cytometric evaluation showed that both extracts promoted apoptosis, with the n-hexane extract producing the highest apoptotic rates in both cell lines. SK-MEL-30 cells displayed greater sensitivity to apoptosis induction than U87 cells. The combined findings suggest that DNA damage and apoptosis contribute to the anticancer activity of *H. rhamnoides* fruit extracts.

Keywords: *Hippophae rhamnoides*, U87, SK-MEL-30, MTT, comet, annexin V

ÖZ

Hippophae rhamnoides L., yaygın olarak yalancı iğde olarak bilinen, besinsel ve tıbbi özellikleri nedeniyle Doğu Avrupa ve Asya'da geleneksel olarak kullanılan bir bitkidir. Yalancı iğde meyvesi, iyi belgelenmiş antioksidan özelliklere sahip biyoaktif bileşikler bakımından zengindir; ancak özellikle agresif kanser türlerindeki antikanser potansiyeli halen yeterince araştırılmamıştır. Glioblastoma ve melanom, yüksek mortalite oranlarına sahip en tehlikeli kanser türleri arasında sınıflandırılmaktadır. Bu çalışmada, yalancı iğde meyvelerinin %80 etanol ve n-hekzan ekstrelerinin insan glioblastoma (U87) ve melanom (SK-MEL-30) hücre hatlarındaki sitotoksik, genotoksik ve apoptotik etkileri araştırılmıştır. Hücreler, 0,05–0,6 mg/mL konsantrasyon aralığında ekstrelerle muamele edilmiştir. Sitotoksikite MTT testi kullanılarak değerlendirilmiş, genotoksikite Comet testi ile belirlenmiş ve apoptoz Annexin V/PI akım sitometrisi ile analiz edilmiştir. Her iki ekstre de U87 ve SK-MEL-30 hücrelerine karşı anlamlı sitotoksik aktivite göstermiştir; ancak yanıtın büyüklüğü konsantrasyona, maruz kalma süresine ve ekstraksiyon çözücüsüne bağlı olarak değişiklik göstermiştir. n-Hekzan ekstresi, özellikle uzun süreli maruziyet sonrasında, etanolü ekstreye kıyasla genel olarak daha güçlü antiproliferatif etkiler göstermiştir. Comet testi analizleri, ekstre ile muamele edilen hücrelerde kontrollere kıyasla anlamlı düzeyde artmış DNA hasarı olduğunu ortaya koymuş ve bu durum genotoksik stresin induklendiğine işaret etmiştir. Akış sitometrisi değerlendirmeleri, her iki ekstre de hücreleri apoptoza yönlendirdiğini, n-hekzan ekstresinin ise her iki hücre hattında en yüksek apoptotik oranları oluşturduğunu göstermiştir. SK-MEL-30 hücreleri, apoptoz indüksiyonuna karşı U87 hücrelerine göre daha yüksek duyarlılık göstermiştir. Elde edilen bulgular birlikte değerlendirildiğinde, DNA hasarı ve apoptozun *H. rhamnoides* meyve ekstrelerinin antikanser aktivitesine katkıda bulunduğunu göstermektedir.

Anahtar Kelimeler: *Hippophae rhamnoides*, U87, SK-MEL-30, MTT, comet, anneksin V

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INTRODUCTION

Currently, cancer remains a leading cause of global morbidity and mortality, with malignant melanoma and glioblastoma multiforme representing two of the most aggressive and treatment-resistant forms of the disease (Orzan et al., 2023; Yalamarty et al., 2023). Deriving from a common embryological precursor, the neuroectoderm, these two malignancies exhibit high invasive potential and complex resistance mechanisms that pose significant hurdles for clinical management (Orzan et al., 2023). The limited efficacy of conventional approaches, including surgical resection, chemotherapy, and radiotherapy, in substantially improving survival rates has driven the scientific community to seek more effective, specific, and less toxic alternative strategies (Yalamarty et al., 2023). In this context, integrating the advancements of modern medicine with the rich bioactive components provided by plants has become increasingly critical, particularly in understanding the potential of plant-derived therapeutics to overcome the resistance of these lethal cancers (Yalamarty et al., 2023; Sevim-Tatar, Secerli, & Gdl Bacanlı, 2025; Yang et al., 2025).

Herbal extracts provide promising formulations for cancer prevention and treatment. Researchers have demonstrated that plant extracts, their isolated fractions, or individual components can inhibit enzymes that stimulate cancer cells, stimulate the production of anticancer enzymes, enhance the immune system response, and protect DNA and other structural cellular molecules through their antioxidant effects (Deshmukh & Sakarkar, 2011; Liaudanskas, Žvikas, & Petrikaitė, 2021). In Traditional Chinese Medicine and Traditional Korean Medicine, adjuvant therapy with natural products is often used because it may reduce adverse effects, overcome drug resistance, and improve survival rates (An et al., 2025; Lee, Seong, & Won, 2021).

Hippophae rhamnoides L., commonly known as sea buckthorn, is a deciduous shrub belonging to the Elaeagnaceae family and has been traditionally used in Eastern Europe and Asia for its nutritional and me-

dicinal properties. In Trkiye, it is known as “Yalancı iğde,” “Çıçırgan,” “Yabani İğde” or “Karga dikenı” locally (Baytop, 1999; Ercisli, Orhan, Ozdemir, & Sengul, 2007; Hayta, Gulaboglu, & Kutlu, 2021). Sea buckthorn fruits, long used in traditional medicine across China, Tibet, Mongolia, Central Asia, and Russia, are a rich source of bioactive substances (Dong et al., 2020).

Berries that are also listed in the Chinese Pharmacopoeia are rich in bioactive compounds including flavonoids, phenolic acids, carotenoids, vitamins (notably vitamin C and E), unsaturated fatty acids, and phytosterols. These constituents have been associated with a range of pharmacological activities, such as antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, and anticancer activities (Dienaitė et al., 2020; Suryakumar & Gupta, 2011; Visan et al., 2023).

Sea buckthorn extracts have ability to induce apoptosis and inhibit cell proliferation, possibly acting as a therapeutic adjuvant that may reduce the cytotoxic damage commonly seen with standard oncological treatments (Olas, Skalski, & Ulanowska, 2018). Fruits of plants are well known as a good source of dietary antioxidants such as proanthocyanidins, gallocatechins and flavonol glycosides-phenolic compounds that are responsible for their anticancer effects through protecting cells from oxidative damage, which can lead to genetic mutations and carcinogenesis (Dienaitė et al., 2020; Dong et al., 2020; Muscolo, Mariateresa, Giulio, & Mariateresa, 2024).

In recent years, growing attention has been directed toward the anticancer potential of *H. rhamnoides* (sea buckthorn). Several in vitro and in vivo studies have demonstrated that its extracts can inhibit tumor cell proliferation, induce apoptosis, and modulate key signaling pathways involved in carcinogenesis (Masoodi et al., 2020; Olas et al., 2018; Visan et al., 2023; Żuckowski, 2023). Although the anticancer activity of *H. rhamnoides* has been investigated in various cancer models, including breast, colon, prostate, lung, and glioma cancers, studies specifically evaluating the cytotoxic and antiproliferative effects of its fruit extracts

on human melanoma and glioblastoma cells remain limited (Ali et al., 2019; Dvorska et al., 2025; Olas et al., 2018; Sevim-Tatar, Secerli, Gök, & Güdül Bacanlı, 2026; Wang et al., 2022; Żuckowski, 2023).

Regarding melanoma, *H. rhamnoides* fruit extracts have been reported to significantly reduce the viability of the WM793 melanoma cell line (Borczak, Kapusta-Duch, Domagała, & Doskočil, 2023). In contrast, research investigating the effects of sea buckthorn on glioma has predominantly focused on the rat C6 glioma model (Wang et al., 2022). Comprehensive studies employing human-derived glioblastoma cell lines, such as U87, are still scarce. Similarly, investigations involving human melanoma cell lines remain relatively limited. These gaps in the literature indicate that the anticancer potential of *H. rhamnoides* against human melanoma and glioblastoma has not yet been thoroughly explored and warrants further investigation (Olas et al., 2018; Wang et al., 2022).

In this context, the present study aimed to investigate the anticancer potential of *H. rhamnoides* fruit extracts in human glioblastoma (U87) and melanoma (SK-MEL-30) cell lines. Considering the limited number of studies focusing on these cancer models, particular emphasis was placed on evaluating the effects of the extracts on cell viability, DNA damage, and apoptosis-related parameters as an integrated mechanistic evaluation. To our knowledge, this is the first comparative study to assess the cytotoxic, genotoxic, and proapoptotic effects of a Turkish-origin sea buckthorn fruit extracts in U87 glioblastoma and SK-MEL-30 melanoma cells under standardized experimental conditions. Furthermore, the opportunity to compare two different cancer types with different embryological origins in the same experimental setting allows the evaluation of the tumor-selective activity of the extracts. By elucidating the biological responses induced by *H. rhamnoides* extracts, this study seeks to expand current knowledge regarding their anticancer properties and provide further evidence supporting the potential use of sea buckthorn as a promising source of novel bioactive compounds for cancer therapy.

MATERIAL AND METHODS

Chemicals

Dulbecco's Modified Essential Medium (DMEM), fetal bovine serum (FBS), RPMI-1640, trypsin-EDTA (0.25%), phosphate-buffered saline (PBS), and penicillin-streptomycin were purchased from Vivacell (Denzlingen, Germany). N-Lauroylsarcosine sodium salt, Triton X-100, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sodium hydroxide (NaOH), dimethyl sulfoxide (DMSO), normal melting point agarose (NMA), low melting point agarose (LMA) and ethidium bromide (EtBr) were provided from Sigma-Aldrich (St. Louis, Missouri, USA). Annexin V kit was obtained from Serva (Heidelberg, Germany).

Plant material

H. rhamnoides were collected from Erzurum, Türkiye, in September of 2021. The plant was identified using the Flora of Turkey (vol 7) and its taxonomic status was verified using Plants of the World Online (Davis, 1982; Skala, Toma, Kowalczyk, Śliwiński, & Sitarek, 2018). Voucher specimens have been stored under the related GUEF3927 code in the Herbarium of Gazi University, Faculty of Pharmacy, Ankara, Türkiye (GUEF).

Methods

Extraction

The fruits of the plant were air-dried in a cool, well-ventilated, and shaded area, and processed in a lab mill into a fine powder. Independent n-hexane and 80% ethanol extracts were prepared by macerating 10 g of powdered plant material in the respective solvent (3 × 100 mL) for 3 days at room temperature with occasional agitation. The extracts were concentrated to dryness under reduced pressure using a rotary evaporator (Büchi Rotavapor, Switzerland) at 40 °C water bath temperature. The concentrated extracts were subsequently lyophilized in vacuo and stored at +4°C until further analysis. The n-hexane and the ethanolic extracts were dissolved in 1% dimethyl sulfoxide (DMSO) to create

stock solutions at 1 mg/mL for cytotoxicity investigations. The stock solutions were then serially diluted to create working concentrations of 0.05, 0.1, 0.2, 0.4 and 0.6 mg/mL (Sevim-Tatar et al., 2025).

Cell culture

U87 human glioblastoma and SK-MEL-30 human melanoma cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; high glucose, L-glutamine) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in an incubator (Nve, Trkiye) maintained at 37 °C, 5% CO₂, and 95% relative humidity. Cells were used within a defined passage range (passages [15–20]) to ensure experimental consistency. Cell lines were kindly obtained from the Turkish Vaccine Institute Aziz Sancar Research Center. For cytotoxicity assays, cells were seeded into 96-well plates, whereas 6-well plates were used for genotoxicity and apoptosis analyses. Cell numbers were determined using an automated cell counter.

Cytotoxicity determination

Following the procedure described in the Cell Culture section, U87 and SK-MEL-30 cells were seeded into 96-well plates with a cell number of 15000 cell/well and incubated for 24 hours to allow cell attachment. After incubation, the culture medium was removed, and the cells were treated with plant extracts at concentrations of 0.05, 0.1, 0.2, 0.4, and 0.6 mg/mL, prepared in complete medium, with exposure times of 24 and 72 hours.

After the exposure periods, the treatment media were aspirated, and MTT solution (0.5 mg/mL) was added to each well. Following a 4-hour incubation with MTT, plates were gently shaken for 10 minutes, and absorbance was measured at 570 nm using a microplate reader. Medium containing 1% DMSO served as the negative control (100% viability), and viability of extract-treated cells was calculated as (absorbance of treated wells/absorbance of control wells) × 100. IC₅₀ values were determined using dose-response curves. The IC₅₀ values were calculated via GraphPad Prism 8.0 with non-linear regression (Korkmaz, Secerli, ErdoĖan, & Gdl Bacanlı, 2025).

Genotoxicity determination

The alkaline single-cell gel electrophoresis (Comet) assay was used to measure the DNA damage induced by the plant extracts. With a few minor adjustments, the alkaline comet test was carried out in an alkaline environment (pH > 13). Cells were maintained as detailed in the "Cell culture" section, seeded into 6-well plates at a density of 400000/well, incubated at 37 °C for 24 hours, and then trypsinized following PBS washing. After the incubation, the solutions in the wells were discarded, and the cells were subjected to the minimal (0.2 mg/mL) and maximal (0.6 mg/mL) concentrations in the incubator for a duration of 24 hours. Following exposure, cells were subjected to trypsinization. Approximately 10000–15000 cells per slide were obtained by making the required dilutions. The cell suspension was amalgamated with LMA (1%) and applied to slides previously coated with NMA (1%). Coverslips were positioned on the slides, and the agar was permitted to solidify at 4 °C. After solidification of the agar, the coverslips were removed, and the slides were incubated in a cold lysis solution (2.5 M NaCl, 100 mM EDTA, 100 mM Tris, 1% sodium sarcosinate, pH 10.0 with Triton-X-100 and 10% DMSO) for a minimum duration of 1 hour. Following lysis, the slides were incubated for 20 minutes in a cold electrophoresis solution (1 mM sodium EDTA and 300 mM NaOH, pH 13.0) without the application of current. By the end of this period, horizontal electrophoresis was performed at 25 V and 300 mA for 20 minutes. After electrophoresis, the slides were washed with distilled water and neutralized in PBS for 15 minutes. After neutralization, the slides were incubated in 50%, 75%, and 99% ethanol for 5 minutes each, respectively, to eliminate excess water, then permitted to dry at room temperature for at least 24 h. For evaluation of genotoxic effects on cells, EtBr (20 µg/mL) was applied to the slides, and tail intensity (%) values were quantified using a fluorescence microscope (Leica, Germany) with 40x magnification and Perceptive Instruments Comet IV software (United Kingdom), with a minimum of 100 cells per slide.

A medium with 1% DMSO was used as the negative control (Korkmaz et al., 2025; Møller et al., 2020).

Apoptosis analysis

Cells were cultured as detailed in the Cell Culture section, plated at a density of 5×10^5 cells/well in 6-well plates, and incubated at 37 °C for 24 hours. After the incubation, the solutions in the wells were and the cells were subjected to an additional 24 hours with the minimal (0.2 mg/mL) and maximal (0.6 mg/mL) concentrations. After the treatment period, cells were collected via trypsinization and stained with Annexin V-FITC and propidium iodide (PI) in accordance with the manufacturer's protocol. Then flow cytometric analysis (CytoFLEX, Beckman Coulter, USA) was performed. Data analysis was conducted with quadrant gating, with results expressed as the percentage distribution of cells throughout each population. Annexin V-FITC/PI staining patterns, which include viable, early apoptotic, late apoptotic, and necrotic cells, were used to quantify apoptotic cell populations. For every sample, at least 10,000 events were obtained (Korkmaz et al., 2025).

Statistical analysis

SPSS software (version 20.0; SPSS Inc., Chicago, IL) was used to analyse the data. Levene's test was performed to confirm the homogeneity of variances, while the Kolmogorov-Smirnov test was applied to examine the data's normal distribution. Comparisons between groups were performed using Student's t-test or one-way ANOVA, followed by the LSD post hoc procedure where appropriate. Statistical significance was established at $p < 0.05$. All studies were independently replicated three times, each with duplicate samples.

RESULTS AND DISCUSSION

Cytotoxicity

Figure 1 illustrates the effects of *H. rhamnoides* ethanolic and n-hexane extracts on the viability of U87 glioblastoma and SK-MEL-30 melanoma cells at 24 and 72 hours. Both extracts reduced cell viability

in a time- and concentration-dependent manner, though response patterns varied by cell line. After 24 hours of exposure, both extracts decreased U87 cell viability in a dose-dependent manner. At the highest tested concentration, the n-hexane extract proved more cytotoxic (reducing viability to ~30%) than the ethanolic extract (~50%). In contrast, SK-MEL-30 cells displayed a biphasic response; while viability decreased significantly at lower concentrations, it partially recovered at higher doses, particularly with the n-hexane extract. After 72 hours, both cell lines exhibited increased sensitivity. In U87 cells, viability dropped below 20% at 0.1–0.2 mg/mL, though partial recovery occurred at higher concentrations, suggesting a non-linear dose-response. SK-MEL-30 cells followed a similar, more pronounced concentration-dependent trend, with the n-hexane extract demonstrating the strongest antiproliferative activity (reducing viability to ~10% at 0.6 mg/mL) compared to the ethanolic extract (~35–40%). Overall, *H. rhamnoides* fruit extracts possess significant cytotoxic activity against both glioblastoma and melanoma cells, with the n-hexane extract generally exhibiting greater potency. The increased efficacy after 72 hours suggests that the antiproliferative activity is time-dependent, likely attributed to the enrichment of non-polar bioactive constituents that interfere with cellular survival and proliferation pathways. These findings align with previous observations where bioactive phytochemicals, such as fatty acid derivatives and phenolic compounds, were shown to induce pro-apoptotic signaling and ROS modulation in malignant cells (Rehman et al., 2024).

For the ethanolic extract, the same batch used in the present study was previously characterized by HPLC in our previous work. The results indicate that the observed cytotoxic activity of the ethanolic extract may be due, at least in part, to the presence of rutin, protocatechuic acid, quercetin, and quercetin-3-O-glucoside (Sevim-Tatar et al., 2026). Furthermore, the differential susceptibility observed between the two cancer cell lines underscores the potential for

selective toxicity, a phenomenon previously identified in other botanical extracts (uckowski, 2023; Gazizova, Gronbach, Oppermann, Kragl, & Engel, 2025).

The greater biological activity observed for the n-hexane extract is likely attributable to its enrichment in lipophilic phytochemicals that are extracted more efficiently by non-polar solvents. These compounds

may promote mitochondrial membrane depolarization, increase intracellular ROS, facilitate cytochrome c release, and activate the intrinsic apoptotic cascade. The synergistic interactions among these lipophilic constituents may therefore explain the stronger cytotoxic and pro-apoptotic activity of the n-hexane extract compared with the ethanolic fraction.

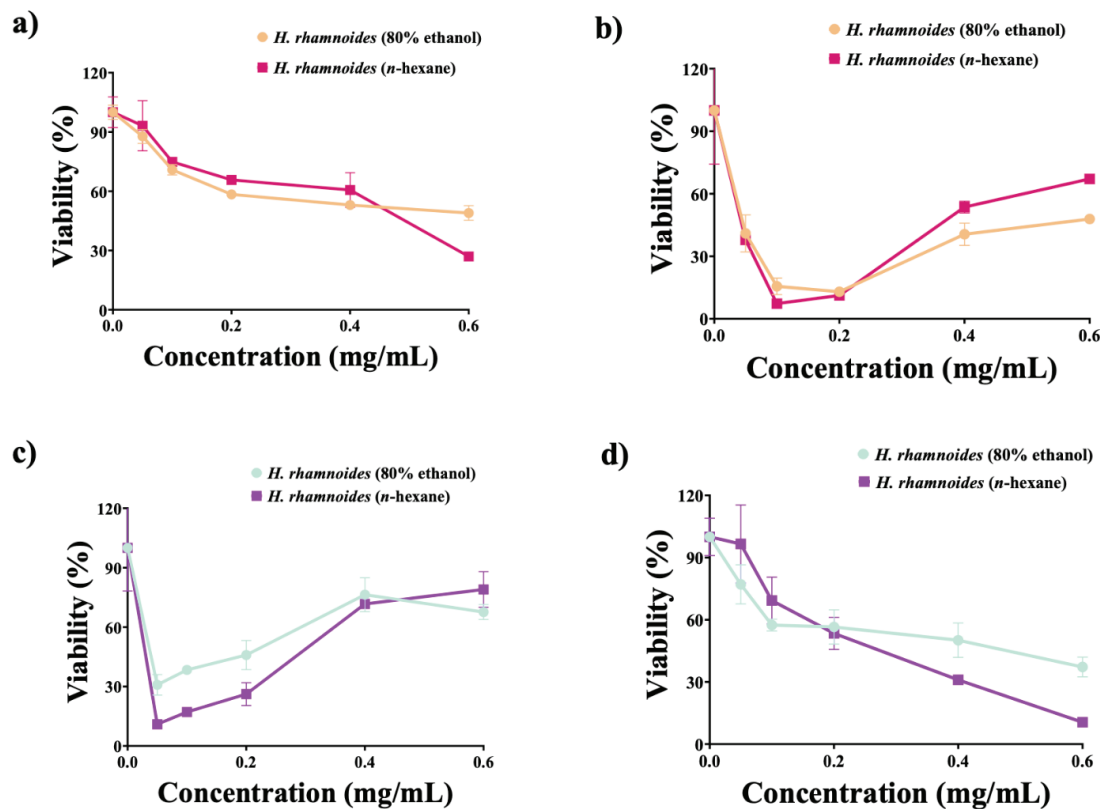


Figure 1. Cytotoxic effects of *H. rhamnoides* ethanolic and n-hexane extracts after 24 hours exposure: a) U87, c) SK-MEL-30 cell lines; after 72 hours exposure: b) U87, d) SK-MEL-30 cell lines. Data were expressed as mean $\pm$ standard deviation

Genotoxicity determination

The genotoxic potential of *H. rhamnoides* ethanolic and n-hexane extracts was assessed in U87 and SK-MEL-30 cells after 24 hours using the alkaline comet assay, with DNA damage quantified by tail intensity. In U87 cells, both extracts induced higher DNA damage compared to the negative control. Peak damage occurred at 0.6 mg/mL for the ethanolic extract and 0.2 mg/mL for the n-hexane extract. Notably, the n-hexane

extract exhibited a non-linear dose-response, as DNA damage decreased when the concentration increased from 0.2 to 0.6 mg/mL, though levels remained above control values. Similarly, treatment with *H. rhamnoides* extracts increased DNA damage in SK-MEL-30 cells, most notably at 0.2 mg/mL n-hexane. While variations between treatment groups were modest, the results confirm that *H. rhamnoides* extracts induce DNA damage in both cell lines after 24 hours, sug-

gesting that genotoxicity contributes to the observed anticancer activity (Figure 2.). The observed increase in DNA damage in both U87 and SK-MEL-30 cells may represent one of the mechanisms underlying the cytotoxic activity of *H. rhamnoides* extracts (Balıkcı et al., 2020). Induction of DNA strand breaks can activate DNA damage response pathways, leading to cell cycle arrest and apoptosis in cancer cells. However,

the non-linear response observed particularly in U87 cells suggests that the genotoxic effects may depend on the concentration-dependent balance of multiple bioactive constituents present in the extracts rather than a simple dose-dependent mechanism. This observation is consistent with the complex phytochemical composition of sea buckthorn fruits and warrants further mechanistic investigation (Wang et al., 2022).

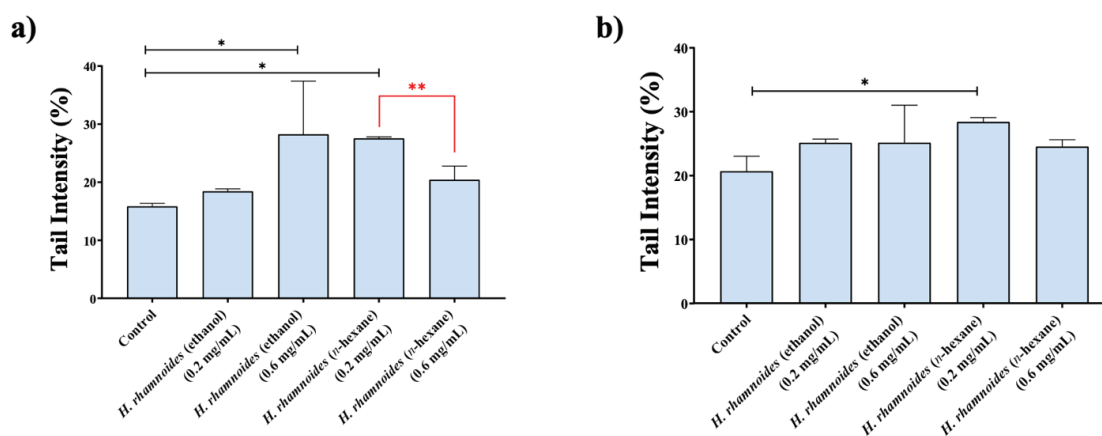


Figure 2. Genotoxicity assessment of *H. rhamnoides* ethanolic and n-hexane extracts in a) U87, b) SK-MEL-30 cell lines after 24 hours exposure; * compared to negative control ($p < 0.05$); ** compared between groups ($p < 0.05$). Data were expressed as mean $\pm$ standard deviation

The non-linear dose-response observed in the present study may reflect the complex nature of crude plant extracts, which contain numerous bioactive compounds with distinct molecular targets and biological activities. At lower concentrations, pro-apoptotic and DNA-damaging constituents may exert their effects more efficiently, whereas at higher concentrations interactions among phytochemicals, activation of cellular defense mechanisms, or the rapid elimination of severely damaged cells may reduce the measurable level of DNA damage (Bacanlı, Aydın, Başaran, & Başaran, 2017). Similar biphasic or hormetic dose-response patterns have been reported for several phytochemical-rich plant extracts and are considered characteristic of complex natural mixtures rather than purified compounds. Therefore, the observed non-linear response likely reflects the combined effects of multiple constituents acting through different biological pathways.

Apoptosis analysis

The induction of apoptosis was corroborated by the substantial increase in cell populations within the Annexin V+/PI+ and Annexin V+/PI− quadrants, denoting a preferential progression toward programmed cell death as opposed to necrosis. This shift toward controlled apoptotic pathways, rather than passive necrotic cell death, is significant as it prevents the uncontrolled release of intracellular contents that could otherwise trigger inflammation in the surrounding microenvironment (Abdel-Rasol, Al-Hamadani, Ibrahim, & El-Sayed, 2025).

We evaluated apoptosis induction in U87 glioblastoma cells following treatment with *H. rhamnoides* extracts using Annexin V-FITC/PI staining and flow cytometry (Figure 3.). Representative dot plots revealed distinct changes in cell population distributions after

treatment. The control group consisted primarily of viable cells, with minimal apoptotic levels. Treatment with the ethanolic extract at 0.2 mg/mL resulted in only a slight increase in the apoptotic population compared to the control, with no further enhancement observed at higher concentrations. Conversely, the n-hexane extract markedly induced apoptosis in U87 cells. Exposure to 0.2 mg/mL of the n-hexane extract reduced cell viability from 89.05% to 72.27% while increasing both early and late apoptotic populations, representing the highest overall apoptotic rate among the tested groups. While treatment with

0.6 mg/mL of the n-hexane extract also significantly decreased cell viability and increased apoptotic fractions, the effect was slightly less pronounced than that observed at 0.2 mg/mL. Overall, these flow cytometric findings demonstrate that the n-hexane extract possesses a greater capacity to induce apoptosis in U87 cells than the ethanolic extract. The concurrent increase in both early and late apoptotic populations suggests the activation of programmed cell death pathways, supporting apoptosis as a primary mechanism of the anticancer activity of *H. rhamnoides* against glioblastoma cells.

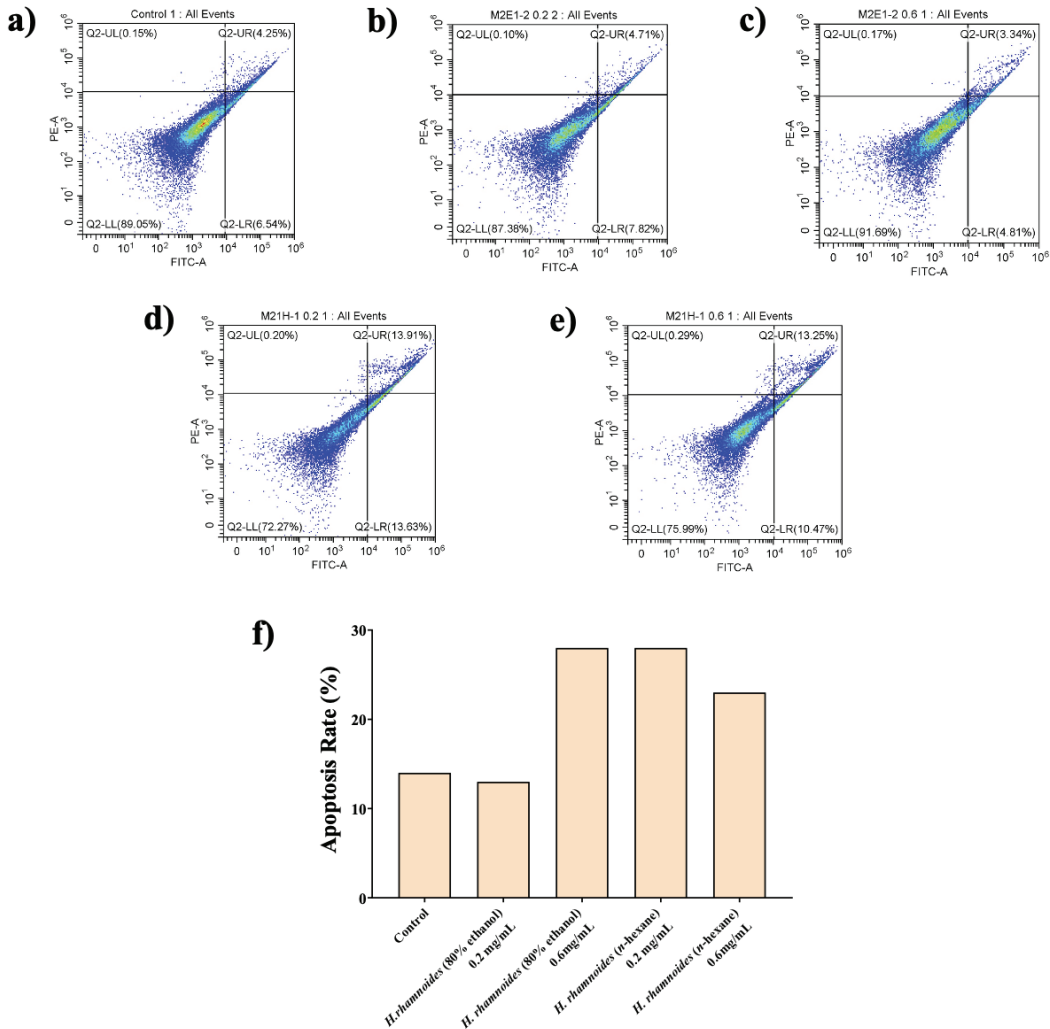


Figure 3. Flow cytometric analysis of apoptosis in U87 cells. Cells stained with Annexin V-FITC and PI. a) Negative control (1% DMSO), b) *H. rhamnoides* ethanolic extract (0.2 mg/mL), c) *H. rhamnoides* ethanolic extract (0.6 mg/mL), d) *H. rhamnoides* n-hexane extract (0.2 mg/mL), e) *H. rhamnoides* n-hexane extract (0.6 mg/mL), f) Quantification of live, early apoptotic, late apoptotic, and necrotic cell populations. The dot plots show quadrants representing necrotic (Q1, upper left), late apoptotic (Q2, upper right), early apoptotic (Q3, lower right), and live (Q4, lower left) cells

As seen in Figure 4, the apoptotic effects of *H. rhamnoides* ethanolic and n-hexane extracts on SK-MEL-30 melanoma cells were evaluated using flow cytometry. The control group displayed high viability and minimal apoptosis. Treatment with 0.2 mg/mL ethanolic extract increased the total apoptotic rate fourfold compared to the control, reducing cell viability to 75.02%; however, this effect diminished at 0.6 mg/mL, reflecting a non-linear dose-response. The n-hexane extract demonstrated superior pro-apoptotic efficacy, notably at 0.2 mg/mL, where it reduced cell viability to 70.93% and induced the highest overall apoptotic rate. These results suggest that apoptosis, via programmed cell death pathways, is a primary mechanism of cytotoxicity for *H. rhamnoides* extracts in melanoma cells, with the n-hexane fraction showing greater potency. These observations align with previous studies demonstrating that specific plant-derived fractions can significantly augment apoptotic rates in malignant lines (Fayed, Aboulela, & Refaey, 2022; Weerapreeyakul, Nonpunya, Barusrux, Thitimetharoch, & Sripanidkulchai, 2012), confirming that cell death induction remains a robust biomarker for evaluating the therapeutic efficacy of natural products. The n-hexane fraction of *H. rhamnoides* fruit is

enriched in lipophilic constituents -carotenoids, triterpenoids (ursolic and oleanolic acids), phytosterols (β -sitosterol, stigmasterol), tocopherols, and unsaturated fatty acids- each previously linked to anticancer activity (Olas et al., 2018; Żuchowski, 2023). The observed cytotoxicity in U87 and SK-MEL-30 cells is consistent with reports on sea buckthorn low-polarity fractions where triterpenoids were identified as the main bioactive class responsible for cancer-selective effects (Marciniak, Kontek, Żuchowski, & Stochmal, 2020). Interestingly, a direct mechanistic rationale for our findings in SK-MEL-30 cells has been reported for ursolic and oleanolic acids as inhibitors of proliferation and inducers of apoptosis in melanoma cells such as SK-MEL-2 (Caunii et al., 2017). The DNA damage revealed by the comet assay is consistent with the pro-oxidant β -sitosterol and carotenoid activity occurring preferably in cancer cells with elevated basal ROS levels (Baeza-Morales et al., 2024; Shin, Song, Oh, Keum, & Saini, 2020; Zhao, Chang, Qu, Li, & Cui, 2009). The apoptotic response probably represents the caspase-3/7 activation induced by the synergistic action of these lipophilic constituents, as previously demonstrated for sea buckthorn extracts (Marciniak et al., 2020; Visan et al., 2023).

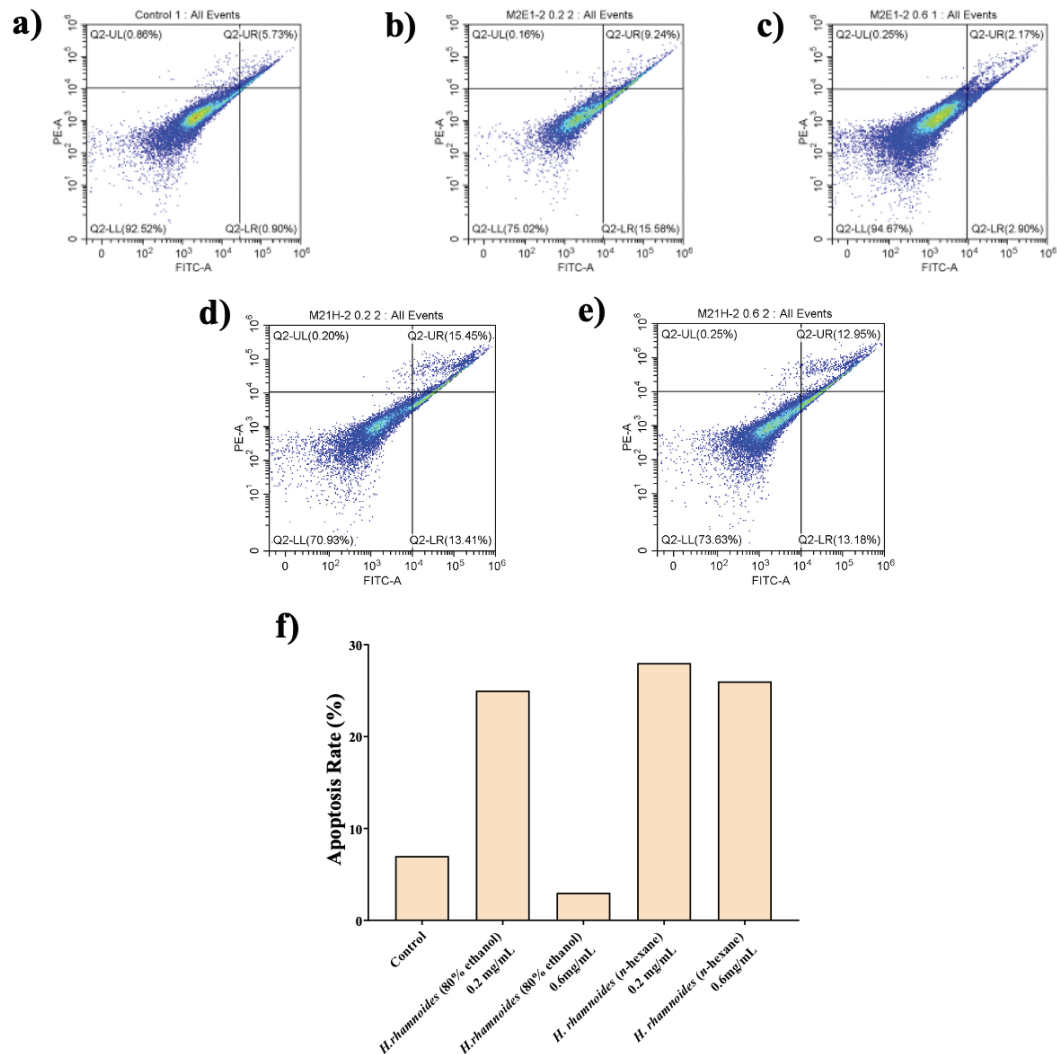


Figure 4. Flow cytometric analysis of apoptosis in SK-MEL-30 cells. Cells stained with Annexin V-FITC and PI. a) Negative control (1% DMSO), b) *H. rhamnoides* ethanolic extract (0.2 mg/mL), c) *H. rhamnoides* ethanolic extract (0.6 mg/mL), d) *H. rhamnoides* n-hexane extract (0.2 mg/mL), e) *H. rhamnoides* n-hexane extract (0.6 mg/mL), f) Quantification of live, early apoptotic, late apoptotic, and necrotic cell populations. The dot plots show quadrants representing necrotic (Q1, upper left), late apoptotic (Q2, upper right), early apoptotic (Q3, lower right), and live (Q4, lower left) cells

CONCLUSION

The present study demonstrates that *Hippophae rhamnoides* fruit extracts possess significant anticancer activity against human glioblastoma and melanoma cell lines. Both ethanolic and n-hexane extracts reduced cell viability, induced DNA damage, and promoted apoptotic cell death; however, the magnitude of these effects varied according to the specific

extract type, concentration, and cell line tested. Notably, among the tested extracts, the n-hexane fraction generally exhibited superior biological activity, yielding more substantial reductions in cell viability and higher apoptotic rates, particularly in SK-MEL-30 melanoma cells. In parallel, comet assay findings revealed significant increases in DNA damage following treatment, suggesting that genotoxic stress may serve

as a key driver of the observed antiproliferative effects. Complementing these results, flow cytometric analyses confirmed that apoptosis acts as a primary mechanism underlying the cytotoxicity of *H. rhamnoides* extracts, with melanoma cells displaying a more pronounced apoptotic response compared to their glioblastoma counterparts. Interestingly, several endpoints exhibited non-linear dose-response patterns; this phenomenon suggests that the biological activity of *H. rhamnoides* extracts may be governed by the complex interactions of multiple phytochemical constituents rather than simple dose-dependence. Consequently, these findings highlight the necessity of further phytochemical characterization and mechanistic studies to identify the specific compounds responsible for these anticancer effects. Overall, these results provide novel evidence that *H. rhamnoides* fruit extracts, particularly the n-hexane fraction, exert cytotoxic, genotoxic, and pro-apoptotic effects in human glioblastoma and melanoma cells. Ultimately, this study expands the current understanding of the anticancer potential of sea buckthorn and underscores its promise as a source of bioactive compounds for future therapeutic strategies.

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AUTHOR CONTRIBUTION STATEMENT

Investigation, formal analysis, methodology, validation, data curation, visualization, writing –original draft, writing – review & editing; visualization (DST); Methodology, validation; formal analysis (JS); Conceptualization, methodology, supervision, funding acquisition, formal analysis, writing – original draft, writing – review & editing, visualization (MGB).

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

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