

Research Article

Open access

Comparative Effects of Turkish Rose Hip (*Rosa canina* L.) Extracts with Different Polarities on the Healthy and Cancer Cell Lines

Duygu Sevim-Tatar¹, Jülide Secerli², Merve GÜDÜL Bacanlı^{2*}¹Department of Pharmacognosy, Gülhane Faculty of Pharmacy, University of Health Sciences Turkey, Ankara, Türkiye²Department of Pharmaceutical Toxicology, Gülhane Faculty of Pharmacy, University of Health Sciences Turkey, Ankara, Türkiye

DOI: 10.31383/ga.vol9iss2ga06

Abstract

Cancer remains one of the most challenging health problems worldwide, and natural products continue to represent a promising source for safer and more selective anticancer agents. *Rosa canina* L. (rose hip), traditionally used in Türkiye for its antioxidant and anti-inflammatory properties, contains diverse bioactive compounds, including phenolics, carotenoids, and tocopherols, which may contribute to its anticancer potential. This study aimed to compare the cytotoxic, genotoxic, and apoptotic effects of *R. canina* fruit extracts with different polarities (ethanolic and *n*-hexane) on human breast (MCF-7) and colorectal (HT-29) cancer cell lines, as well as their non-tumoral counterparts (MCF-10A and FHC). Extracts were prepared from fruits collected in Tokat, Türkiye, and applied to cells at 0.2-1.0 mg/mL for 24-72 h. Cytotoxicity (MTT assay), DNA damage (comet assay), and apoptosis (Annexin V-FITC/PI flow cytometry) were evaluated in the cells. Both extracts exhibited dose- and time-dependent cytotoxicity, while healthy cells were less affected. The ethanolic extract induced pronounced apoptosis in HT-29 cells at 0.2 mg/mL, whereas the *n*-hexane extract was more effective in MCF-7 cells at 0.6 mg/mL. No significant genotoxicity was observed in healthy cells, suggesting an antigenotoxic profile. Overall, *R. canina* extracts displayed selective anticancer effects primarily through apoptosis rather than DNA damage, indicating their potential as natural sources for the development of complementary therapeutic agents against breast and colon cancers.

***Correspondence**E-mail:
merve.gudulbacanli@sbu.edu.tr**Received**

October, 2025

Accepted

December, 2025

Published

December, 2025

Copyright: ©2025 Genetics & Applications, The Official Publication of the Institute for Genetic Engineering and Biotechnology, University of Sarajevo**Keywords***Rosa canina*, rose hip, anticancer activity, MCF-7, HT-29

Introduction

Cancer remains one of the leading causes of mortality worldwide (Jiménez et al., 2016). Although substantial progress has been achieved in early diagnosis, surgical interventions, and chemotherapeutic strategies, challenges such as drug resistance, adverse side effects, and disease recurrence continue to impede effective treatment across various cancer types. In recent years, the remarkable chemical diversity, multitargeted mechanisms of action, and generally favorable safety profiles of natural products have attracted increasing attention in cancer research (Turan et al., 2018; Yang et al., 2025).

Species of the genus *Rosa* produce rose hips, which are pseudocarps (false fruits) containing a single seed enclosed within a hard achene and surrounded by a fleshy outer layer (Cheikh-Affene et al., 2013; Nadpal et al., 2016). Rose hips are renowned for having one of the highest vitamin C contents among fruits and vegetables, ranging from 300 to 4,000 mg per 100 g (Ercisli, 2007). Traditionally, they have been widely utilized in ethnomedicine for the prevention and treatment of various ailments, including the common cold, influenza, diabetes, arthritis, poor peripheral circulation, and disorders of the gastrointestinal, renal, and lower urinary tracts (Chrubasik et al., 2008; Nadpal et al., 2016; Ögüt, 2022).

Rosa canina L., commonly known as dog rose, is a perennial shrub belonging to the genus *Rosa* within the family Rosaceae, which comprises over 200 species distributed across Europe, Asia, the Middle East, Africa, and North America (Nadpal et al., 2016; Khazaei et al., 2020). The species is widely distributed throughout Türkiye (Ercisli, 2007; Kasapoğlu et al., 2020) and holds significant ethnobotanical importance. Various traditional

products are prepared from its fruits and roots, including rose hip marmalade, rose hip pestil, rose hip syrup, and rose hip tea (Ercisli and Guleryuz, 2005; Ercisli 2007).

Considering phytochemical investigations of *R. canina* hips, many phytochemicals have been identified, including vitamin C, tocopherols, phenolics, bioflavonoids, carotenoids, organic acids, and essential fatty acids (Ercisli, 2007; Demir et al., 2014; Selahvarzian et al., 2018). *R. canina* L. has long been used worldwide as a source of vitamins, medicinal supplements, and food for its nutritional and medicinal benefits (Jiménez et al., 2016; Selahvarzian et al., 2018).

Investigations showed that the *R. canina* hips exhibit a wide range of bioactivities, such as anti-inflammatory (Deliorman Orhan et al., 2007; Jäger et al., 2007; Wenzig et al., 2008), antioxidant (Barros et al., 2011; Demir et al., 2014; Taneva et al., 2016; Peña et al., 2023), antiproliferative (Tumbas et al., 2012; Guimarães et al., 2014; Jiménez et al., 2016; Zhong, 2017; Selahvarzian et al., 2018; Turan et al., 2018; Berköz et al., 2019; Khazaei et al. 2020; Kilinc et al., 2020), anti-obesity, and anti-diabetic activity (Chrubasik et al., 2008; Orhan et al., 2009; Fattahi et al., 2017; Mourabit et al., 2023).

A limited number of studies have explored the anticancer potential of *Rosa canina* (Tumbas et al., 2012; Jiménez et al., 2016; Zhong, 2017; Selahvarzian et al., 2018; Turan et al., 2018; Berköz et al., 2019; Kilinc et al., 2020; Khazaei et al. 2020). Moreover, a review of the literature indicates that investigations focusing on the anticancer efficacy of *R. canina* species cultivated in Türkiye are particularly scarce (Turan et al., 2018; Kilinc et al., 2020).

Thus, the present study aimed to evaluate and compare the cytotoxic, genotoxic, and apoptotic

effects of *n*-hexane and ethanolic extracts of *Rosa canina* L. fruits on human breast (MCF-7) and colorectal (HT-29) cancer cell lines, in parallel with non-cancerous human mammary epithelial (MCF-10A) and colonic epithelial (FHC) cells.

Material and methods

Apparatus

Absorbance measurements for cytotoxicity analyses were performed using an Allsheng microplate reader (Hangzhou, China). Cell counting was conducted with a NanoEntek Cell Counter (Seoul, Korea). Flow cytometry analysis was performed using a Beckman Coulter (Brea, CA, USA). The graphs were drawn with GraphPad Prism 8.0.2.

Chemicals

Dulbecco's Modified Essential Medium (DMEM), phosphate-buffered serum (PBS), trypsin-EDTA (0.25%), penicillin-streptomycin, fetal bovine serum (FBS) and RPMI-1640 were purchased from Vivacell (Denzlingen, Germany). Normal melting point agar (NMA), low melting point agar (LMA), sodium hydroxide (NaOH), dimethyl sulfoxide (DMSO), ethidium bromide (EtBr), PBS tablets, N-Lauroylsarcosine sodium salt, Triton X-100, ethanol, MTT and 2',7'-Dichlorofluorescein diacetate (DCF-DA) were purchased from Sigma Aldrich (St. Louis, Missouri, USA). Annexin V kit was obtained from Serva (Heidelberg, Germany).

Plant material

The fresh fruits of *R. canina* were collected in September 2023 in Tokat, Türkiye. The Flora of Turkey (Vol. 4) and current literature were used to

identify the plant (Nilsson 1972; Bozhuyuk et al., 2021). Voucher specimens have been kept in the Herbarium of Hacettepe University, Faculty of Pharmacy, Ankara, Türkiye (HUEF) under the associated HUEF24243 code.

Preparation of Extracts

The plant's fruits were air-dried in a cool, shaded, well-ventilated area, then ground into a fine powder using a laboratory mill. For extraction, powdered rose hips (10 g for each extract) were macerated with *n*-hexane (3 × 100 mL) and 80% aqueous ethanol (3 × 100 mL) at room temperature. The resulting extracts were concentrated to dryness under reduced pressure and subsequently lyophilized *in vacuo*. For cytotoxicity studies, the ethanolic extract was dissolved in distilled water, whereas the *n*-hexane extract was dissolved in 1% dimethyl sulfoxide (DMSO) to obtain stock solutions at 1 mg/mL. Working concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/mL were then prepared by serial dilution of the stock solutions (Faur et al., 2024).

Cell Culture

MCF-10A, HT-29, and MCF-7 cells were cultured in DMEM (with high glucose, L-glutamine) medium containing 10% FBS and 1% penicillin-streptomycin; FHC cells were cultured in RPMI-1640 medium containing 10% FBS and 1% penicillin-streptomycin in an incubator with 5% CO₂ and 95% humidity at 37 °C. After the cells reached a sufficient number, they were lifted from the bottom of the flask with trypsin-EDTA (0.25%). Trypsin was deactivated with the serum in complete medium that was added for necessary dilutions and cells were seeded onto different plates according to the relevant analyses. An

automatic cell counter was used to determine cell numbers. Cell culture equipment used, cell numbers and related analyses are given in Table 1.

Table 1. Cell culture equipment, cell numbers and related analyses

Cell Culture Equipment	Cell Number	Related Analysis
96 well plate	$1,5 \times 10^3$	Cytotoxicity
6 well plate	5×10^5	Genotoxicity Apoptosis

Cytotoxicity Determination

FHC, HT-29, MCF-10A, and MCF-7 cells were cultured as described under the heading “cell culture” and seeded onto plates at the numbers indicated in Table 1. The cells seeded on the plates were incubated in the incubator for 24 hours. After the incubation period, the medium in the wells was discarded. Plant extracts were added at 0.2-1 mg/mL, diluted in the medium, and the cells were exposed to the extracts for 24 and 72 hours. Following the exposure period, the extract solutions in the wells were discarded, and MTT solution (5 mg/mL) was added to each well. The cells were then incubated with this solution for 4 hours at 37 °C. After the exposure period, the solutions in the wells were discarded, and a 100 µL of DMSO solution was added to each well and the plate shaken in shaker for 5 minutes. The color intensity of the wells was measured at 570 nm. Medium containing 1% DMSO was used as a negative control. Cell viability was calculated using the formula below:

$$\text{Viability} = (\text{Absorbance of test well} / \text{Absorbance of negative control well}) \times 100$$

Inhibitor concentration (IC_{50}) values were determined based on dose-response curves (Korkmaz et al., 2025).

Selectivity Index Calculation

The selectivity index (SI) was calculated to evaluate the preferential cytotoxicity of *R. canina* extracts toward cancer cells compared to their healthy cells. The SI value was determined as the ratio of the IC_{50} obtained for healthy cells to that for cancer cells, according to the following equation:

$$SI = \frac{IC_{50} (\text{healthy cells})}{IC_{50} (\text{cancer cells})}$$

$SI > 1 \rightarrow$ The extract is more selective towards cancer cells (more toxic than healthy cells).

$SI \approx 1 \rightarrow$ The selectivity is weak.

$SI < 1 \rightarrow$ The extract is more toxic towards healthy cells.

Genotoxicity Determination

DNA damage caused by the extracts in cells was assessed using the alkaline single-cell gel electrophoresis (Comet) method. Cells were cultured as described under the title “cell culture,” seeded at the indicated numbers onto the plates listed in Table 1, and incubated at 37°C for 24 hours. Following incubation, the solutions in the wells were discarded, and the cells were exposed to the lowest (0.2 mg/mL) and highest (0.6 mg/mL) concentrations below the IC_{50} values in the incubator for 24 hours. Following exposure, cells were trypsinised. The necessary dilutions were made to get approximately 10,000-15,000 cells per slide. The cell suspension was mixed with LMA (1%) and spread onto slides previously

coated with NMA (1%). Coverslips were placed on the slides, and the agar was allowed to solidify at 4°C. After the agar solidified, the coverslips were discarded, and the slides were incubated in 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 at least 1 hour. Following lysis, the slides were incubated in cold electrophoresis solution (1 mM sodium EDTA and 300 mM NaOH, pH 13.0) for 20 minutes without applying current. At the end of this period, horizontal electrophoresis was performed for 20 minutes at 25 V and 300 mA. After electrophoresis, the slides were neutralized in PBS for 15 minutes. After neutralisation, the slides were incubated in 50%, 75%, and 99% ethanol for 5 minutes each, respectively, to remove excess water, then allowed to dry at room temperature for at least 24 hours. To evaluate genotoxic effects on cells, EtBr (20 µg/mL) was applied to the slides, and tail intensity (%) values were measured under a fluorescence microscope (Leica (Germany) with 40x magnification using Perceptive Instruments Comet IV software (United Kingdom), with at least 100 cells per slide. A medium containing 1% DMSO was used as a negative control (Korkmaz et al., 2025).

Apoptosis Analysis

Cells were maintained as detailed in the “Cell Culture” section, plated at the specified cell densities onto the corresponding plate formats shown in Table 1, and incubated at 37 °C for 24 h. After incubation, the culture medium was removed, and the cells were treated for an additional 24 h with the lowest (0.2 mg/mL) and highest (0.6 mg/mL) concentrations selected below the IC₅₀ value. At the end of the treatment period, cells were harvested by trypsinisation and stained

with Annexin V–FITC and propidium iodide (PI) following the manufacturer’s protocol. Flow cytometric assessment was then performed, using dot-plot gating to resolve four cell populations: viable (Annexin⁻/PI⁻), early apoptotic (Annexin⁺/PI⁻), late apoptotic (Annexin⁺/PI⁺), and necrotic (Annexin⁻/PI⁺). Data analysis was carried out based on quadrant gating, and results were presented as the percentage distribution of cells in each population (Korkmaz et al., 2025).

Statistical Analysis

Data analysis was performed using SPSS software (version 20.0; SPSS Inc., Chicago, IL). The Kolmogorov–Smirnov test was applied to examine the normal distribution of the data, while Levene’s test was used to verify the homogeneity of variances. Group comparisons were conducted using either Student’s t-test or one-way ANOVA, followed by the LSD post hoc procedure where appropriate. Differences were considered statistically significant at $p < 0.05$. All experiments were repeated independently three times, each with duplicate samples.

Results and Discussion

Currently, nearly 60% of anticancer drugs are derived from naturally occurring plant-based compounds (Turan et al., 2018). Due to their efficacy against cancer cells and relative safety toward normal cells, natural products have emerged as promising candidates in the development of novel anticancer therapeutics (dos Santos Júnior et al., 2010). Nevertheless, studies investigating the cytotoxic potential of *Rosa canina* remain scarce (Tumbas et al., 2012; Guimarães et al., 2013; Jiménez et al., 2016; Zhong, 2017; Turan et al., 2018; Berkosz et al.,

2019; Kilinc et al., 2020). In one study, the methanolic extract of *R. canina* from Portugal exhibited cytotoxicity against human lung, colon, cervical, and liver cancer cell lines, but not against breast cancer cells (Guimarães et al. 2013). Therefore, this study evaluated the cytotoxic effects of ethanolic and *n*-hexane extracts of *R. canina* on healthy and cancer cells. Figure 1 illustrates the cytotoxic effects of *R. canina* ethanol and *n*-hexane extracts on healthy (FHC) and cancerous (HT-29) colon cell lines after 24 hours and 72 hours of exposure. A clear time- and concentration-dependent decrease in cell viability was observed in all cell lines, with a more pronounced effect for the *n*-hexane extract. In both exposure periods, HT-29 colorectal cancer cells were the most sensitive to treatment. The *n*-hexane extract showed a steep decline in viability at concentrations above 0.25 mg/mL, with survival below 20% at higher doses. This effect intensified over time, suggesting a delayed cytotoxic action

possibly related to the lipophilic constituents that accumulate within cellular membranes or organelles. In contrast, the ethanol extract caused a milder, more gradual reduction in viability, consistent with its polar composition rich in phenolic and flavonoid compounds that may exert cytostatic rather than directly cytotoxic effects. Healthy colon epithelial cells (FHC) demonstrated greater tolerance, maintaining viability above 50% at intermediate concentrations, particularly with ethanol extract. This difference indicates a potential selective toxicity favouring cancer cell inhibition while sparing normal epithelial cells. Collectively, the data suggest that *R. canina*, particularly its *n*-hexane fraction, exerts time-dependent, selective cytotoxicity against colorectal cancer cells, supporting its potential as a source of non-polar anticancer phytochemicals. Our results are consistent with other studies with *R. canina* (Tumbas et al., 2012; Jiménez et al., 2016; Zhong, 2017; Turan et al., 2018).

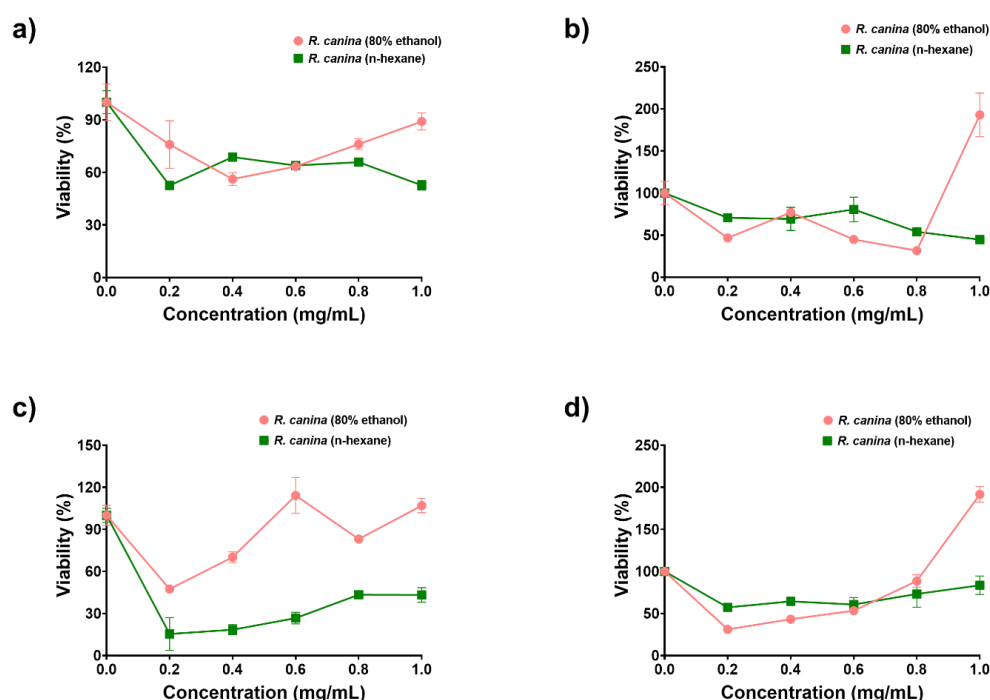


Figure 1. Cytotoxic effects of *R. canina* extracts in colorectal FHC (non-cancer) and HT-29 (cancer) cell line: a) FHC after 24 hours exposure, b) FHC after 72 hours exposure, c) HT-29 after 24 hours exposure, d) HT-29 after 72 hours exposure.

Besides, Figure 2 illustrates the viability responses of healthy breast epithelial noncancer (MCF-10A) and breast cancer (MCF-7) cell lines following 24 hours and 72 hours exposure to *R. canina* ethanol and *n*-hexane extracts. Both extracts induced measurable cytotoxicity in a concentration- and time-dependent manner, yet with distinct intensity patterns between healthy and malignant cells. In MCF-10A cells, both extracts exhibited relatively mild cytotoxic effects. After 24 hours of exposure, cell viability remained above 70% even at higher concentrations, particularly with the ethanol extract, indicating limited toxicity toward non-transformed epithelial cells. Prolonged exposure to 72 hours resulted in a modest decline in viability, more pronounced with the *n*-hexane extract, suggesting that the non-polar bioactive components may exert cumulative effects over time. Nonetheless, the overall response pattern demonstrates a favorable

cytocompatibility profile of *R. canina* toward normal mammary cells. Conversely, MCF-7 cancer cells displayed greater sensitivity to both extracts, especially at extended incubation times. The *n*-hexane extract caused a marked, dose-dependent reduction in viability, with viability decreasing below 40% at the highest tested concentration after 72 hours. The ethanol extract produced a more moderate decrease, consistent with its phenolic-rich composition that may primarily trigger growth inhibition or oxidative imbalance rather than direct membrane damage. Collectively, these results suggest that *R. canina*, particularly its *n*-hexane fraction, exerts selective cytotoxicity toward MCF-7 cancer cells while maintaining minimal impact on normal MCF-10A cells. The selective action may arise from differences in membrane composition, metabolic rate, or redox homeostasis between healthy and malignant breast epithelial cells.

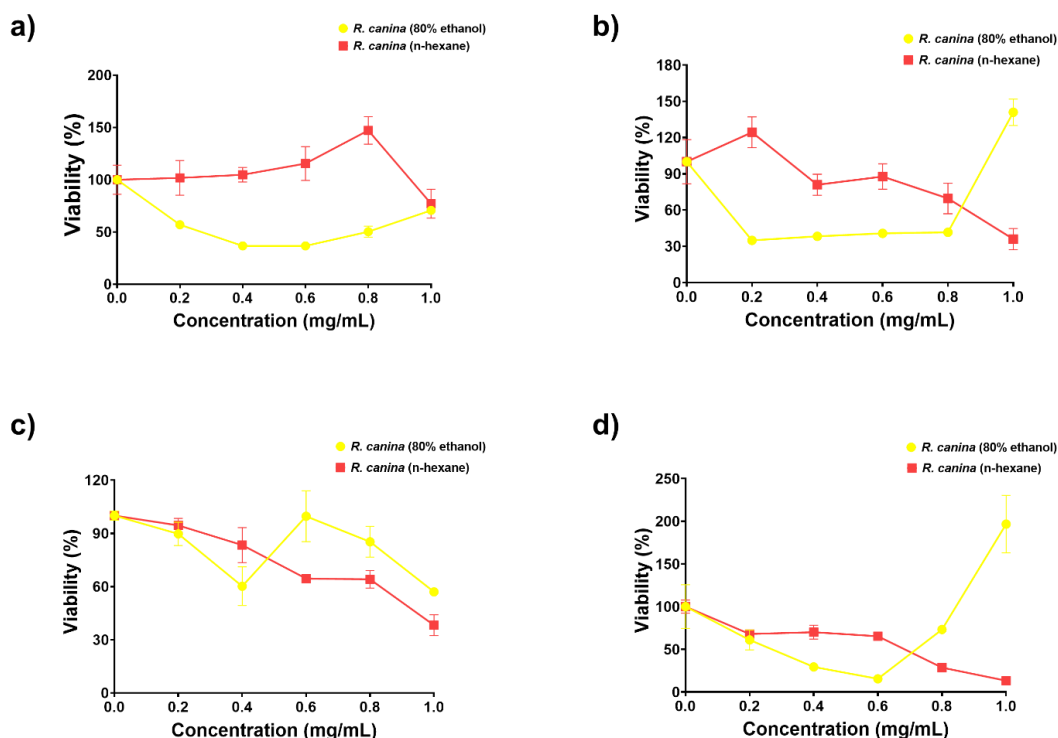


Figure 2. Cytotoxic effects of *R. canina* extracts in breast non-cancer MCF-10A and cancer MCF-7 cell line: a) MCF-10A after 24 hours exposure, b) MCF-10A after 72 hours exposure; c) MCF-7 after 24 hours exposure, d) MCF-7 after 72 hours exposure.

The cytotoxic potential of *R. canina* ethanol and *n*-hexane extracts was assessed on healthy (FHC and MCF-10A) and cancerous (HT-29 and MCF-7) cell lines after 24 and 72 hours of incubation. The obtained IC₅₀ values revealed a distinct pattern of dose- and time-dependent cytotoxicity that varied with both the extract type and the cell line. At 24 hours, the ethanol extract exhibited moderate cytotoxicity, with IC₅₀ values ranging from 0.305 mg/mL (HT-29) to 3.066 mg/mL (MCF-7). Notably, HT-29 colorectal cancer cells were the most sensitive, while MCF-7 breast cancer cells showed considerable resistance. After 72 hours, a moderate increase in cytotoxicity was observed in healthy FHC cells (IC₅₀ = 0.575 mg/mL). In contrast, a slight reduction was observed in HT-29 cells (IC₅₀ = 0.728 mg/mL), suggesting possible cellular adaptation or metabolic tolerance over prolonged exposure. The ethanol extract maintained low toxicity toward healthy cells (FHC and MCF-10A), indicating an acceptable selectivity profile. In contrast, the *n*-hexane extract demonstrated stronger, time-dependent cytotoxicity, particularly against HT-29 cells, with the IC₅₀ decreasing from 0.787 mg/mL at 24 hours to 0.178 mg/mL at 72 hours. This pronounced sensitivity suggests a significant accumulation or delayed action of non-polar bioactive constituents such as triterpenes. The cytotoxic effect on healthy FHC and MCF-10A cells was comparatively milder, with IC₅₀ values remaining above 0.9 mg/mL at 72 hours. The extract also induced moderate toxicity on MCF-7 cells (IC₅₀ = 0.673 mg/mL), indicating a broader yet still selective anticancer profile (Table 2). Overall, *R. canina* extracts, particularly the *n*-hexane fraction, exhibited preferential cytotoxicity toward HT-29 colorectal cancer cells while sparing non-cancerous epithelial cells. These findings suggest that non-polar constituents of *R. canina*

may exert selective anticancer activity, potentially through mechanisms involving membrane disruption or mitochondrial dysfunction.

The SI values further confirmed the preferential cytotoxicity of *R. canina* extracts toward malignant cells. At 24 hours, both ethanol and *n*-hexane extracts demonstrated notable selectivity for HT-29 colorectal cancer cells (SI = 3.95 and 3.73, respectively), indicating stronger selectivity for cancer cells than healthy cells. After 72 hours, the ethanol extract lost its selectivity (SI = 0.79). In contrast, the *n*-hexane fraction maintained and even enhanced its selectivity (SI = 5.30), reflecting a time-dependent accumulation of cytotoxic activity specific to the cancer phenotype. In breast cells, the SI values were generally lower. The ethanol extract showed minimal selectivity toward MCF-7 cells (SI < 0.3), consistent with its weak cytotoxicity. In contrast, the *n*-hexane extract displayed moderate selectivity (SI = 1.79 at 24 hours and 1.31 at 72 hours), suggesting a mild preferential cytotoxicity against breast cancer cells while preserving MCF-10A viability. Overall, these results indicate that *R. canina*, especially its *n*-hexane extract, exerts remarkable selectivity toward colorectal cancer cells, highlighting the potential of its non-polar bioactive constituents as promising leads for cancer-targeted phytotherapeutic development (Table 3).

Figure 3 presents the genotoxic responses of normal (FHC) and cancerous (HT-29) colon cells following exposure to ethanol and *n*-hexane extracts of *R. canina*. In FHC cells, neither extract induced substantial DNA damage compared with the negative control. Although a slight elevation in tail intensity was observed, the differences did not reach statistical significance ($p > 0.05$). These results indicate that both extracts exhibit minimal genotoxic potential toward healthy colon epithelial cells, consistent with the relatively high IC₅₀ values

observed in the cytotoxicity assays. In contrast, HT-29 colorectal cancer cells exhibited a significant, dose-dependent increase in DNA damage following exposure to both extracts ($p < 0.05$ vs. control). The *n*-hexane extract produced a more pronounced effect, resulting in approximately a 2-fold increase in tail intensity relative to the control, suggesting enhanced induction of DNA strand breaks. The ethanol extract also induced measurable genotoxicity, albeit to a lesser extent, suggesting that both polar and non-polar phytochemicals contribute to genomic stress in malignant cells. The inter-group comparison ($p < 0.05$) confirmed that

HT-29 cells were substantially more susceptible to extract-induced genotoxicity than FHC cells. Taken together, these findings demonstrate that *R. canina* extracts, particularly the *n*-hexane fraction, can selectively induce DNA damage in cancerous colon epithelial cells. This selective genotoxic stress may be associated with differential redox homeostasis and DNA repair capacities between normal and malignant cells. The results reinforce the hypothesis that *R. canina* contains bioactive compounds that elicit anticancer genotoxic mechanisms without compromising the genomic stability of healthy tissue.

Table 2. Inhibitor concentration (IC_{50}) values *R. canina* extracts in colon and breast cells

	IC_{50} (mg/mL)							
	24 hours				72 hours			
	FHC cells	HT-29 cells	MCF-10A cells	MCF-7 cells	FHC cells	HT-29 cells	MCF-10A cells	MCF-7 cells
<i>R. canina</i> (ethanol)	1.205	0.305	0.805	3.066	0.575	0.728	0.929	4.984
<i>R. canina</i> (<i>n</i> -hexane)	2.938	0.787	1.683	0.942	0.943	0.178	0.885	0.673

Table 3. Selectivity Index (SI) values of *R. canina* extracts in colon and breast cells

Extract	Exposure Time	FHC/HT-29	MCF-10A/MCF-7
<i>R. canina</i> (ethanol)	24 hours	3,95	0,26
	72 hours	0,79	0,19
<i>R. canina</i> (<i>n</i> -hexane)	24 hours	3,73	1,79
	72 hours	5,30	1,31

Figure 4 presents the genotoxicity profiles of *R. canina* ethanol and *n*-hexane extracts in healthy (MCF-10A) and cancerous (MCF-7) breast cell lines. In MCF-10A cells, both extracts caused minimal DNA damage compared to the negative control, with no statistically significant differences ($p > 0.05$). The ethanol extract, in particular, exhibited negligible genotoxicity, suggesting a protective or

DNA-stabilizing effect of its phenolic constituents. These findings imply that *R. canina* extracts are largely non-genotoxic to non-malignant breast epithelial cells, consistent with their low cytotoxicity observed in viability assays. In contrast, MCF-7 breast cancer cells showed significantly higher tail intensity values after treatment with both extracts ($p < 0.05$ vs. control),

indicating the induction of DNA strand breaks. The effect was more pronounced with the *n*-hexane extract, resulting in a markedly higher level of DNA damage than with the ethanol extract ($p < 0.05$ between groups). This observation aligns with the extract's more potent cytotoxicity, suggesting that its non-polar constituents may facilitate oxidative or apoptotic DNA fragmentation in cancer cells. The

apparent difference in DNA damage between MCF-10A and MCF-7 cells highlights the selective genotoxicity of *R. canina*, which preferentially targets malignant cells while sparing normal ones. As in our study, the antigenotoxic effects of *R. canina* extracts have also been demonstrated in previous studies (Kasimoglu and Uysal, 2015; Johnson et al., 2022).

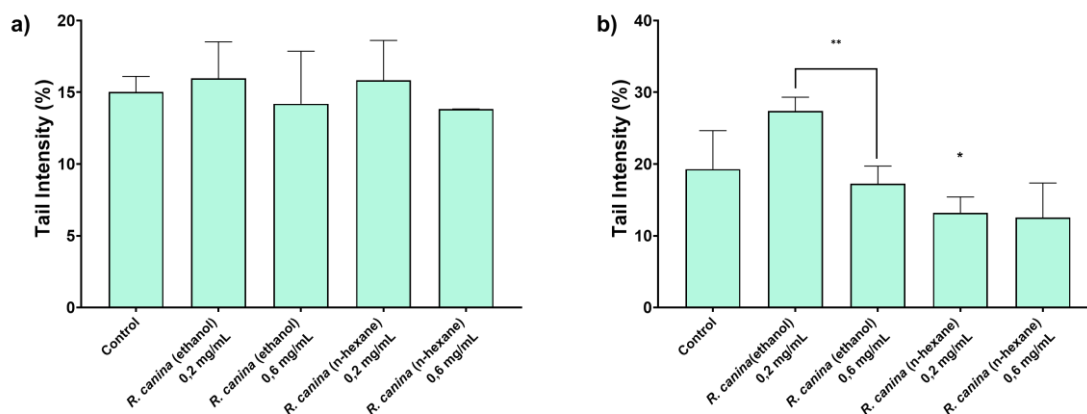


Figure 3. Genotoxicity assessment of *R. canina* ethanol and *n*-hexane extracts in colorectal cells: a) non-cancer FHC cells, b) cancer HT-29 cells after 24 hours exposure; * compared to negative control (control treated with 1% DMSO) ($p < 0.05$); ** compared between groups

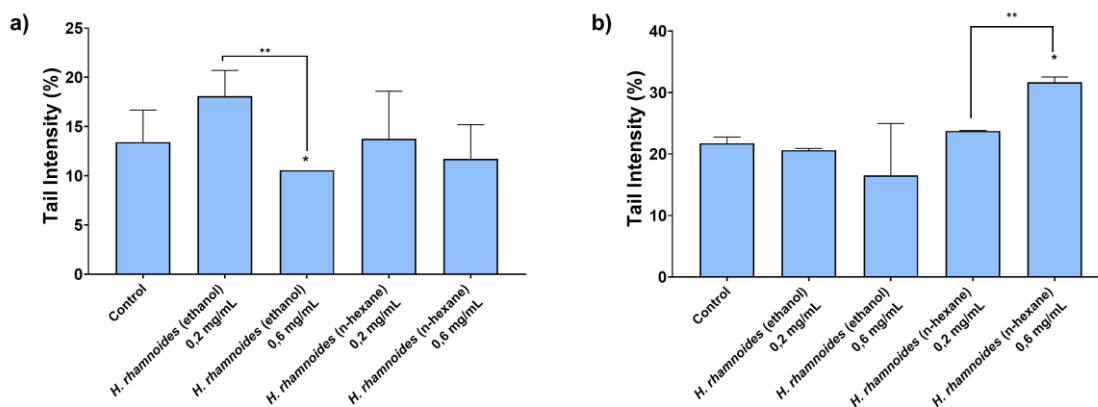


Figure 4. Genotoxicity assessment of *R. canina* extracts in a) MCF-10A cells, b) MCF-7 cells after 24 hours exposure; * compared to negative control ($p < 0.05$); ** compared between groups

It was also observed that a low concentration of ethanolic extract (0.2 mg/mL) in colon cancer cells significantly reduced the number of viable cells and induced apoptosis, compared to other concentrations and *n*-hexane extracts (Figure 5).

Moreover, in breast cancer cells, a high concentration (0.6 mg/mL) of the *n*-hexane extract reduced the number of viable cells. It increased the number of apoptotic cells compared to other extracts (Figure 6). While these results are similar

to those of studies using *R. canina* extracts (Mármol et al., 2019; Kilinc et al., 2020), our study is particularly valuable for comparing the effects of the extraction solvents on apoptotic activity. These results suggest that the extract's ability to induce apoptosis varies depending on the type of cancer.

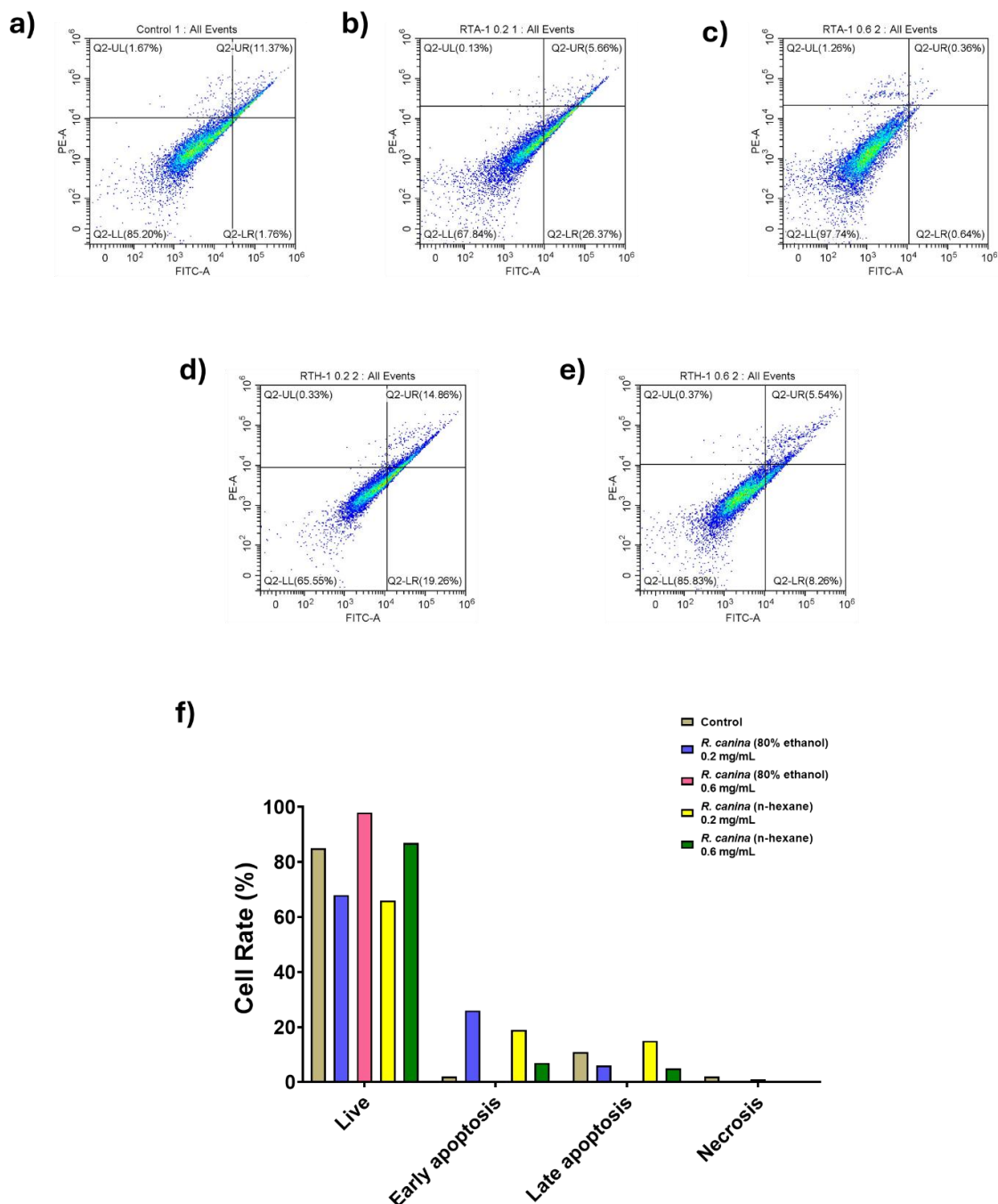


Figure 5. Flow cytometric analysis of apoptosis in HT-29 cells. Cells stained with Annexin V-FITC and PI. a) Negative control (1% DMSO), b) *R. canina* ethanolic extract (0.2 mg/mL), c) *R. canina* ethanolic extract (0.6 mg/mL), d) *R. canina* n-hexane extract (0.2 mg/mL), e) *R. canina* n-hexane extract (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.

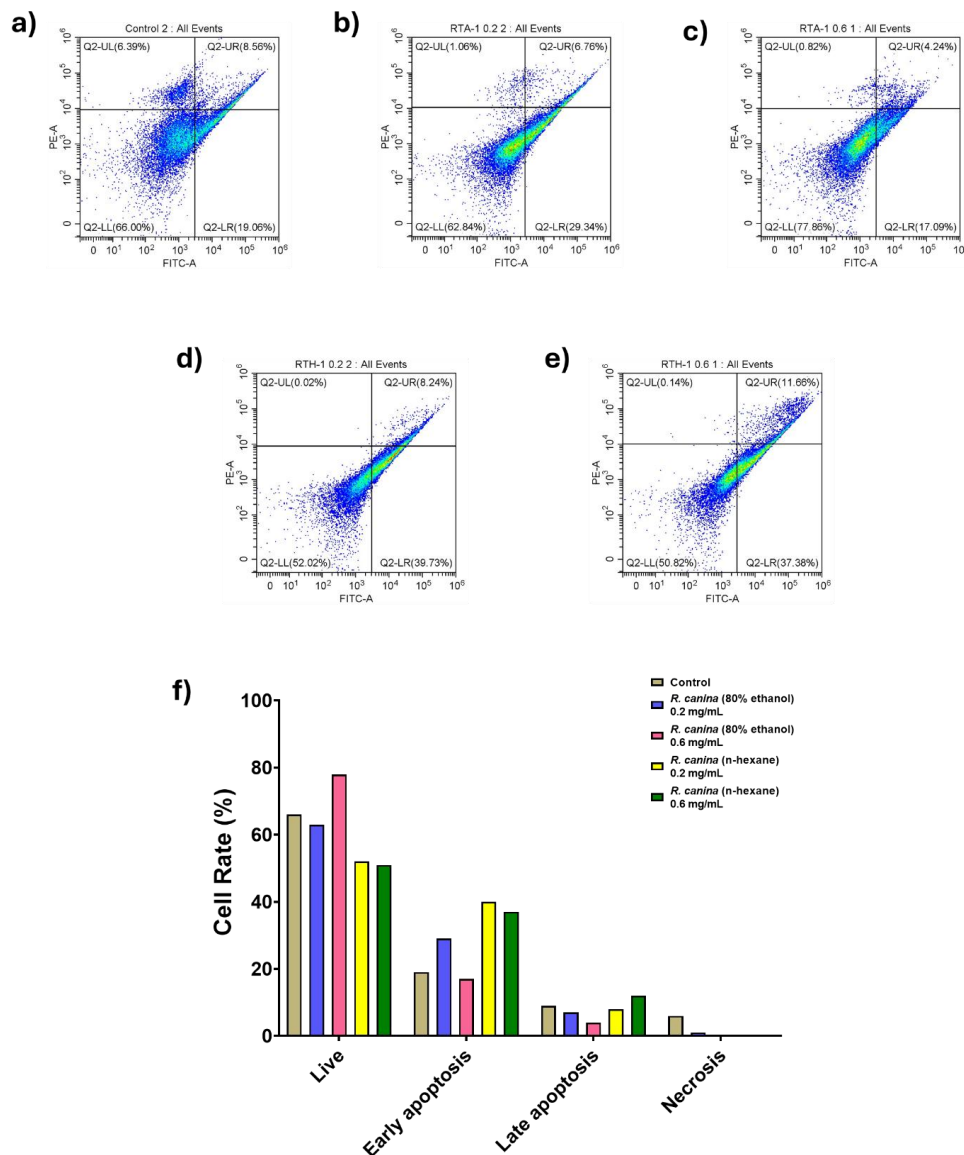


Figure 6. Flow cytometric analysis of apoptosis in MCF-7 cells. Cells stained with Annexin V-FITC and PI. a) Negative control (1% DMSO), b) *R. canina* ethanolic extract (0.2 mg/mL), c) *R. canina* ethanolic extract (0.6 mg/mL), d) *R. canina* *n*-hexane extract (0.2 mg/mL), e) *R. canina* *n*-hexane 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 increasing incidence of cancer is now driving people to seek alternative treatment methods. Interest in natural therapies is growing, particularly to avoid the side effects of traditional chemotherapy drugs. This study evaluated the impact of two

extracts (ethanolic and *n*-hexane) of *Rosa canina*, a plant thought to have anticancer activity and grown in Türkiye, on the two most common cancer types (colon and breast), as well as on healthy cells from these cancers. Both ethanol and *n*-hexane fractions

demonstrated measurable anticancer potential; however, their biological profiles diverged substantially, depending on polarity and phytochemical composition. The *n*-hexane extract showed the most potent and selective cytotoxicity, particularly against HT-29 colorectal adenocarcinoma cells. These findings strongly suggest that non-polar bioactive compounds contribute to the extract's selective anticancer mechanism, potentially by activating apoptotic signalling pathways. In contrast, the ethanol extract displayed moderate cytotoxicity and limited selectivity, with genotoxic and apoptotic effects less pronounced. Its activity may be attributed to phenolic and flavonoid constituents capable of eliciting cytostatic rather than directly cytotoxic actions, possibly through regulation of redox balance or inhibition of proliferative signalling. Overall, *R. canina*, and particularly its non-polar fraction, demonstrates promising selective anticancer activity characterized by controlled induction of apoptosis and minimal genotoxicity in healthy cells. These results highlight the plant's potential as a natural source of bioactive compounds for the development of selective, low-toxicity anticancer agents. However, further studies, such as 3D cell cultures and *in vivo* studies, are clearly needed to confirm these effects.

Acknowledgements

The authors would like to thank Health Institutes of Türkiye (TÜSEB) Aziz Sancar Research Center for assistance in supplying MCF-7, HT-29, MCF-10A and FHC cell lines.

Authors' contributions

Duygu Sevim-Tatar:

Investigation; Formal analysis; Methodology; Validation; Data curation; Visualization; Writing –

Original Draft; Writing – Review & Editing; Visualization.

Jülide Secerli:

Methodology; Validation; Formal analysis.

Merve Güdül Bacanlı:

Conceptualization; Methodology; Supervision; Funding acquisition; Formal analysis; Writing – Original Draft; Writing – Review & Editing; Visualization.

Conflict of interest

No potential conflict of interest was reported by the author(s).

References

- Barros L, Carvalho AM, Ferreira, ICFR (2011) Exotic fruits as a source of important phytochemicals: Improving the traditional use of *Rosa canina* fruits in Portugal. Food Research International 44: 2233–2236.
- Berköz M, Yılmaz FO, Hunt AO, Yildirim M, Allahverdiyev O, Aslan A (2019) *Rosa canina* L. ethanolic extract induces the antiproliferative and apoptosis potential in MCF-7 and MDA-MB-468 cell lines. Fresenius Environ Bull 28(5): 3718-3725.
- Bozhuyuk MR, Ercisli S, Karatas N, Ekiert H, Elansary HO, Szopa A (2021) Morphological and biochemical diversity in fruits of unsprayed *Rosa canina* and *Rosa dumalis* ecotypes found in different agroecological conditions. Sustainability 13(14): 8060.
- Cheikh-Affene ZB, Haouala F, Trabelsi N, Boulaaba M, Ksouri R, Harzallah-Skhiri F (2013) Pomological description and chemical composition of rose hips gathered on four *Rosa* species section Caninae growing wild in Tunisia. International Journal of Agricultural Science and Technology 1: 43–50.
- Chrubasik C, Roufogalis BD, Müller-Ladner U, Chrubasik S (2008) A systematic review on the *Rosa canina* effect and efficacy profiles. Phytotherapy Research 22: 725–733.

- Deliorman Orhan D, Hartevioglu A, Küpeli E, Yesilada E (2007) In vivo anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina* L. fruits. *Journal of Ethnopharmacology* 112: 394–400.
- Demir N, Yildiz O, Alpaslan M, Hayaloglu AA (2014) Evaluation of volatiles, phenolic compounds and antioxidant activities of rose hip (*Rosa* L.) fruits in Turkey. *LWT-Food Science and Technology* 57: 126–133.
- Dos Santos Júnior HM, Oliveira DF, de Carvalho D A, Pinto JMA, Campos VAC, Mourão ARB, Pessoa C, de Moraes MO, Costa-Lotufo LV (2010). Evaluation of native and exotic Brazilian plants for anticancer activity. *Journal of Natural Medicines* 64(2): 231-238.
- Ercisli S, Güleriyüz M (2004) Rose hip utilization in Turkey. In: (eds) I International Rose Hip Conference 690.
- Ercisli S. (2007) Chemical composition of fruits in some rose (*Rosa* spp.) species. *Food Chemistry* 104(4): 1379-1384.
- Fattahi A, Niyazi F, Shahbazi B, Farzaei MH, Bahrami G (2017) Antidiabetic mechanisms of *Rosa canina* fruits: an in vitro evaluation. *Journal of Evidence-Based Complementary & Alternative Medicine* 22(1): 127-133.
- Faur CA, Zăhan M, Bunea CI, Hârșan E, Bora FD, Bunea A (2024) Antiproliferative and biochemical evaluation of rose extracts: impact on tumor and normal skin cells. *Frontiers in Plant Science* 15: 1477243.
- Guimarães R, Barros L, Dueñas M, Carvalho AM, Queiroz MJR, Santos-Buelga C et al (2013) Characterisation of phenolic compounds in wild fruits from Northeastern Portugal. *Food Chem* 141(4):3721–30.
- Guimarães R, Barros L, Calhelha RC, Carvalho AM, Queiroz MJRP, Ferreira ICFR (2014) Bioactivity of different enriched phenolic extracts of wild fruits from northeastern Portugal: A comparative study. *Plant Foods for Human Nutrition* 69: 37–42.
- Jäger AK, Eldeen IMS, van Staden J (2007) COX-1 and -2 activity of rose hip. *Phytotherapy Research* 21: 1251–1252.
- Jiménez Alonso S, Gascón S, Luquin Martínez A, Laguna M, Ancín Azpilicueta C, Rodríguez Yoldi MJ (2016) *Rosa canina* extracts have antiproliferative and antioxidant effects on Caco-2 human colon cancer. *PloS one* 11(7): e0159136.
- Johnson W, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, Marks JG, Shank RC, Slaga TJ, Snyder PW, Gill LJ, Heldreth B (2022) Safety assessment of *Rosa canina*-derived ingredients as used in cosmetics. *International Journal of Toxicology* 41(1): 44S-60S.
- Kasapoğlu KN, Altın G, Farooqi AA, Salehi B, Setzer WN, Sharifi-Rad J (2020) Anti-proliferative, genotoxic and cytotoxic effects of phytochemicals isolated from Anatolian medicinal plants. *Cellular and Molecular Biology*: 66(4), 145-159.
- Kasimoglu C, Uysal H (2015) Mutagenic biomonitoring of pirethroid insecticides in human lymphocyte cultures: Use of micronuclei as biomarkers and recovery by *Rosa canina* extracts of mutagenic effects. *Pharmaceutical Biology* 53(5): 625-629.
- Khazaei M, Khazaei M, Pazhouhi M 2020. An overview of the therapeutic potentials of *Rosa canina*: a traditionally valuable herb. *WCRJ* 7:e1580.
- Kilinc K, Demir S, Turan I, Mentese A, Orem A, Sonmez M, Aliyazicioglu Y (2020) *Rosa canina* extract has antiproliferative and proapoptotic effects on human lung and prostate cancer cells. *Nutrition and cancer* 72(2): 273-282.
- Korkmaz E, Secerli J, Erdoğan H, Güdül Bacanlı M (2025) Cracking Amyloid Toxicity: Naringin rescues neuronal cells in a minimal Alzheimer's model. *ACS Chemical Neuroscience*.
- Mármol I, Jiménez-Moreno N, Ancín-Azpilicueta C, Osada J, Cerrada E, Rodríguez-Yoldi MJ (2019) A combination of *Rosa canina* extracts and gold complex favours apoptosis of Caco-2 cells by increasing oxidative stress and mitochondrial dysfunction. *Antioxidants* 9(1): 17.

- Mourabit Y, El Hajjaji S, Taha D, Badaoui B, El Yadini M, Rusu ME, ... Bourais I (2023) HPLC-DAD-ESI/MS phytochemical investigation, antioxidant, and antidiabetic activities of Moroccan *Rosa canina* L. extracts. *Biocatalysis and Agricultural Biotechnology* 52: 102817.
- Nadpal JD, Lesjak MM, Šibul FS, Anačkov GT, Četojević-Simin DD, Mimica-Dukić NM, Beara IN (2016) Comparative study of biological activities and phytochemical composition of two rose hips and their preserves: *Rosa canina* L. and *Rosa arvensis* Huds. *Food Chemistry* 192: 907-914.
- Nilsson Ö (1972). *Rosa* L. In: Davis PH (ed), *Flora of Turkey and the East Aegean Islands*, Vol. 4. Edinburgh University Press, pp. 106–128.
- Orhan N, Aslan M, Hosbas S, Deliorman OD (2009) Antidiabetic effect and antioxidant potential of *Rosa canina* fruits. *Pharmacognosy Magazine* 5(20).
- Ögüt K. *Rosa canina* L. (2022). In: Dereli FTG, İlhan M, Belwal T (eds) *Novel drug targets with traditional herbal medicines: Scientific and clinical evidence*. Springer, pp. 513-523.
- Peña F, Valencia S, Tereucán G, Nahuelcura J, Jiménez-Aspee F, Comejo P, Ruiz A (2023) Bioactive compounds and antioxidant activity in the fruit of rosehip (*Rosa canina* L. and *Rosa rubiginosa* L.). *Molecules* 28(8): 3544.
- Selahvarzian A, Alizadeh A, Baharvand PA, Eldahshan OA, Rasoulia B (2018) Medicinal properties of *Rosa canina* L. *Herbal Medicines Journal* 77-84.
- Taneva I, Petkova N, Dimov I, Ivanov I, Denev P (2016) Characterisation of rose hip (*Rosa canina* L.) fruit extracts and evaluation of their *in vitro* antioxidant activity. *J Pharmacogn Phytochem* 5(2): 35-38.
- Tumbas VT, Čanadanović-Brunet JM, Četojević-Simin DD, Četković GS, Đilas SM, Gille L (2012) Effect of rosehip (*Rosa canina* L.) phytochemicals on stable free radicals and human cancer cells. *Journal of the Science of Food and Agriculture* 92(6): 1273-1281.
- Turan I, Demir S, Kilinc K, Yaman SO, Misir S, Kara H, ... Deger O (2018) Cytotoxic effect of *Rosa canina* extract on human colon cancer cells through repression of telomerase expression. *Journal of Pharmaceutical Analysis* 8(6): 394-399.
- Wenzig E, Widowitz U, Kunert O, Chrubasik S, Bucar F, Knauder E, et al. (2008) Phytochemical composition and *in vitro* pharmacological activity of two rose hip (*Rosa canina* L.) preparations. *Phytomedicine* 15(10):826–35.
- Yang LY, Lei SZ, Xu WJ, Lai Y, Zhang Y, Wang Y, Wang ZL (2025) Rising above: Exploring the therapeutic potential of natural product-based compounds in human cancer treatment. *Tradit Med. Res* 10: 18.
- Zhong L (2017) Anti-cancer effects of bioactive compounds from rose hip fruit in human breast cancer cell lines. *Acta Universitatis Agriculturae Sueciae*.