



Comparative analysis of polar and non-polar extracts of Turkish sea buckthorn fruits and their cytotoxic effects on cancer cell lines

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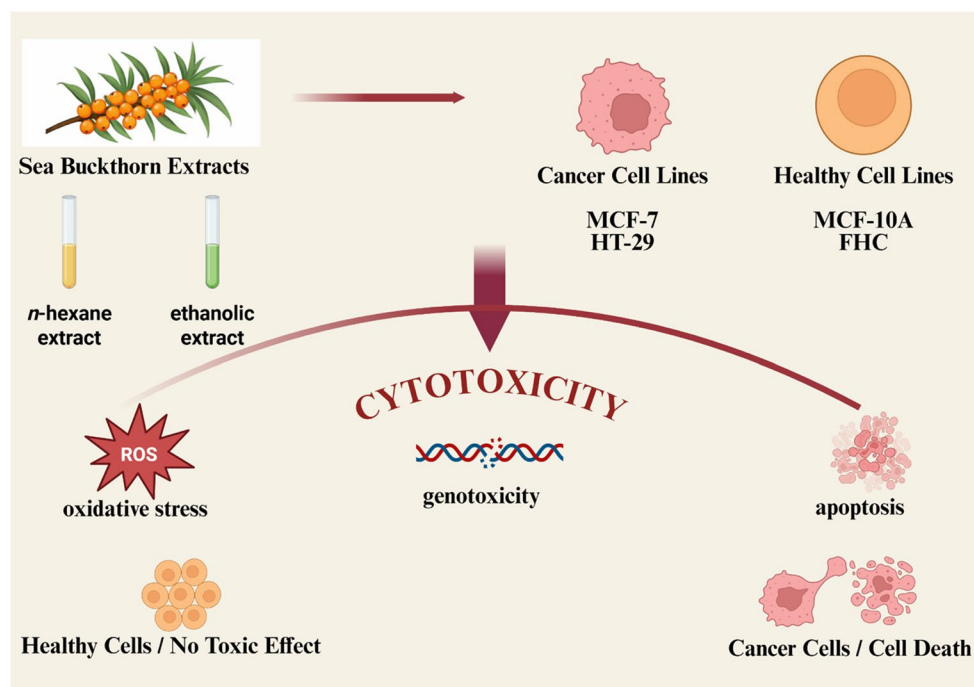
Abstract

Hippophae rhamnoides L., commonly known as sea buckthorn, is a deciduous shrub of the Elaeagnaceae family widely distributed in Europe and Asia. It has gained increasing attention recently due to its nutritional properties and health benefits as food. Berries are a rich source of biologically active constituents, including flavonoids, phenolic acids, carotenoids, tocopherols, phytosterols, vitamins, and unsaturated fatty acids. These compounds have demonstrated a wide range of pharmacological effects. This study evaluated the effects of Turkish sea buckthorn fruit extracts of different polarities on breast (MCF-7) and colorectal (HT-29) cancer cells compared with non-tumorigenic MCF-10 A and FHC cells. According to our results, the ethanolic extract showed stronger cytotoxicity toward cancer cells, with IC₅₀ values of 0.13 and 0.10 µg/mL in MCF-7 cells and 0.18 and 0.076 µg/mL in HT-29 cells after 24 and 72 h, respectively, while showing higher IC₅₀ values in non-tumorigenic counterparts. The highest selectivity index was observed for the ethanolic extract in breast cells after 24 h (SI=11.35). In addition, the extracts increased ROS production up to approximately 2-fold in cancer cells and induced apoptotic cell death, with marked increases in early and late apoptotic populations, particularly in MCF-7 and HT-29 cells. HPLC analysis was conducted to identify phytochemical constituents potentially associated with anticancer activity, because the polar extracts show enhanced selectivity. Considering the results, among the identified phenolic acids and flavonoid compounds, rutin was detected as the predominant constituent (3.3517±0.0317 mg/g), while quercetin, protocatechuic acid, and quercetin-3-*O*-glucoside were also identified in the polar extract.

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Graphical abstract



Keywords *Hippophae rhamnoides* · Cytotoxicity · Reactive oxygen species · RGenotoxicity · Colon cancer · Breast cancer

Introduction

Sea buckthorn (*Hippophae rhamnoides*) fruits, long used in traditional medicine across China, Tibet, Mongolia, Central Asia, and Russia, are a rich source of bioactive substances [1]. Due to their sour and astringent taste, the berries are rarely consumed fresh but have become popular raw materials for jams, drinks, candies, juices, cosmetics, and more. In recent years, their use and processing have attracted growing attention thanks to their nutritional properties and health benefits. Notably, the pharmacological potential of these berries is underscored by their diverse chemical profile, which includes triterpenoids, alkaloids, and flavonoids that exhibit significant anti-tumor activities [2, 3].

Cancer remains a major global health challenge. Despite advances in early detection, surgery, and chemotherapy, drug resistance, side effects, and recurrence continue to pose significant problems across various cancer types. In recent years, natural products have garnered considerable interest in cancer research owing to their chemical diversity, multi-target mechanisms, and generally favorable safety profiles [4, 5]. Specifically, bioactive extracts from sea buckthorn possess the capacity to induce apoptosis and inhibit cellular proliferation, potentially serving as a therapeutic adjuvant that minimizes the cytotoxic damage often associated with

conventional oncological interventions [6]. Sea buckthorn fruits are renowned as a rich source of dietary antioxidants, particularly proanthocyanidins, gallic catechins, and flavonol glycosides-phenolic compounds that underpin their anti-cancer benefits by shielding cells from oxidative damage, which can trigger genetic mutations and carcinogenesis [1, 2, 7]. These traditionally used berries are a rich source of biologically active constituents, including flavonoids, phenolic acids, carotenoids, tocopherols, phytosterols, vitamins, and unsaturated fatty acids. These compounds have demonstrated a wide range of pharmacological effects, such as antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, and anticancer activities [2, 3, 8].

In addition to its pharmacological attributes, sea buckthorn is extensively employed in the food industry, yielding substantial economic value. Renowned for its rich nutritional profile and diverse bioactive compounds -including flavonoids, phenolic acids, carotenoids, and vitamins- it serves as a natural antioxidant and antimicrobial additive across a wide array of food products [6, 9]. Applications have proliferated to encompass sea buckthorn oil, freeze-dried powders, juices, fruit wines, milk tablets, fruit vinegar beverages, teas, preserved fruits, yogurts, and jams. Food industry stakeholders and researchers are presently optimizing their incorporation to enhance the sensory properties

and nutritional quality of these products. Sea buckthorn is therefore widely used by the food industry in the preparation of breads, yogurts, jams, beverages, teas, and other products. Moreover, its leaves hold promises as a functional food that may play a key role in preventing cancer in high-risk populations [10].

H. rhamnoides is generally found wild in Northern and Eastern Anatolia in Türkiye and is known locally as “Yalancı ığde”, ‘Çıçırğan’, ‘Yabani ığde’ or ‘Karga dikenı’ [11–13]. Previous studies on *H. rhamnoides* have primarily focused on phytochemical characterization, antioxidant capacity, and general antiproliferative properties [2, 6, 8, 14–17]. Although anticancer activity has been reported in several experimental models, mechanistic investigations explaining these effects remain limited and have generally evaluated isolated biological endpoints rather than providing an integrated mechanistic assessment. Furthermore, there are no comparative studies on selective cytotoxicity between malignant and healthy cell lines and the effect of extraction polarity on biological activity especially for Turkish sea buckthorn samples. Given the correlation between solvent polarity and the concentration of specific phytochemical constituents, determining the optimal extraction parameters is essential for maximizing the selective anti-proliferative efficacy of *H. rhamnoides* extracts [9, 18]. Additionally, systematic investigations of phytochemical profiling combined with mechanistic endpoints like oxidative stress, genotoxicity and apoptosis are not yet established. Therefore, the present study aimed to address these gaps by comprehensively evaluating polarity-dependent extracts of Turkish sea buckthorn fruits through HPLC-based phytochemical characterization and comparative biological assessment in normal human mammary epithelial (MCF-10A) and colonic epithelial (FHC) cell lines versus cancerous human breast cancer (MCF-7) and colorectal cancer (HT-29) cell lines, with the aim of elucidating their distinct cytotoxic profiles and underlying biological effects.

Materials 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), ethidium bromide (EtBr), PBS tablets, and 2',7'-dichlorofluorescein diacetate (H₂DCF-DA) were

provided from Sigma-Aldrich (St. Louis, Missouri, USA). Annexin V kit was obtained from Serva (Heidelberg, Germany). Gallic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, ellagic acid, fumaric acid, rosmarinic acid, quinic acid, rutin, luteolin-7-O-glucoside, apigenin-7-O-glucoside, hesperidin, myricetin, quercetin, luteolin, apigenin, kaempferol, and naringenin were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Catechin, epicatechin, epigallocatechin, protocatechuic acid, protocatechuic aldehyde, and p-hydroxybenzoic acid were obtained from MedChemExpress (Monmouth Junction, NJ, USA).

Plant material

H. rhamnoides with fully matured fruits collected from Erzurum, Türkiye, in September of 2021. The Flora of Turkey (vol 7) was used to identify the plant [19] and its taxonomic status was verified using Plants of the World Online [20]. Voucher specimens have been kept in the Herbarium of Gazi University, Faculty of Pharmacy, Ankara, Türkiye (GUEF) under an associated GUEF3927 code. The fruits were shade-dried and stored in a dry, shaded environment until extraction.

Methods

Extraction

The fruits of the plant were air-dried in a cool, well-ventilated, and shaded area, and subsequently ground into a fine powder using a laboratory mill. Independent extracts were prepared separately using n-hexane and 80% ethanol solvents. For each extraction procedure, 10 g of powdered plant material was macerated for three consecutive periods of 3 days each at room temperature with 100 mL of solvent several times per day with agitation [21]. The extracts were then filtered through standard filter paper (Whatman No. 1). The filtrates were concentrated to dryness under reduced pressure using a rotary evaporator (Büchi Rotavapor, Switzerland) at 40 °C water bath temperature. The n-hexane extract was evaporated under approximately 250 mbar pressure, while the ethanolic extract was evaporated under approximately 175 mbar pressure. The concentrated extracts were subsequently lyophilized to minimize residual solvent content in vacuo and stored at + 4 °C until further analysis. The ethanolic and n-hexane extracts were obtained with extraction yields of 17.92% and 4.72% (w/w), respectively. A single batch of each extract was prepared and used throughout all biological assays. The n-hexane and the ethanolic extract 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 [4].

Cell culture

Human mammary epithelial cells (MCF-10 A), human breast cancer cells (MCF-7), and human colorectal cancer (HT-29) cell lines were maintained in high-glucose DMEM supplemented with L-glutamine, 10% fetal bovine serum, and 1% penicillin–streptomycin. The human colonic epithelial cell line (FHC) was cultured in RPMI-1640 medium containing 10% FBS and 1% penicillin–streptomycin. All cell lines were cultured under standard conditions at 37 °C in a humidified incubator with 5% CO₂ and ≥ 95% relative humidity. Cells were used within a defined passage range (passages [5–10]) to ensure experimental consistency. Cell lines were kindly obtained from Turkish Vaccine Institute Aziz Sancar Research Center. All cultures were regularly tested for mycoplasma contamination using detection kit, and only mycoplasma-free cells were used in experiments. Following sufficient proliferation, cells were harvested and seeded at a density of 1×10^4 cells/well in 96-well plates for cytotoxicity and reactive oxygen species (ROS) assays, or at 5×10^5 cells/well in 6-well plates for genotoxicity and apoptosis analyses. Cell numbers were determined using an automated cell counter.

Cytotoxicity determination

After seeding the cells in appropriate plates, plant extracts were applied at concentrations of 0.05, 0.1, 0.2, 0.4, and 0.6 mg/mL, with exposure times of 24 and 72 h. Post-exposure, the media containing extracts were discarded, wells were washed with PBS, and MTT solution (5 mg/mL) was added to each well. Following a 4-hour incubation with MTT, absorbance was measured at 570 nm. Cells treated with 1% DMSO served as negative controls (100% viability), and viability of extract-treated cells was calculated as (absorbance of treated wells / absorbance of control wells) $\times$ 100. IC₅₀ values were determined using dose-response curves. The IC₅₀ values were calculated via GraphPad Prism 8.0 with non-linear regression [21]. To evaluate the selectivity of polarity-dependent *Hippophae rhamnoides* extracts, a paired epithelial cell model comprising both malignant and non-malignant counterparts from two tissue types was employed. MCF-7 and MCF-10 A cells were selected to represent breast cancer and non-tumorigenic mammary epithelium, respectively, while HT-29 and FHC cells were used as colon cancer and normal colon epithelial models. This dual-pair design enables direct intra-tissue comparison of cancer versus healthy cells, while also allowing inter-tissue

Fig. 1 Cytotoxic effects of *H. rhamnoides* ethanolic and n-hexane extracts after 24 h exposure (a) MCF-7, c) MCF-10 A e) HT-29, g) FHC cell lines; after 72 h exposure (b) MCF-7, d) MCF-10 A f) HT-29, h) FHC cell lines. Data were expressed as mean $\pm$ standard deviation

validation of the observed effects. Such a model system provides a robust and biologically relevant framework for assessing the selective cytotoxicity of phytochemical extracts across different epithelial origins.

Selectivity index calculation

The selectivity index (SI) was calculated to assess the selective cytotoxicity of *H. rhamnoides* extracts toward cancer cells relative to normal cells. The SI value was computed as the ratio of the IC for normal cells to that for cancer cells, according to the following equation [22]:

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

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

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

SI < 1 → The extract is more toxic towards healthy cells.

Reactive oxygen species (ROS) production

ROS production was assessed using the DCF-DA method. After culturing cells in appropriate plates, the media were discarded and wells washed with PBS. Then, 100 μ L of 0.5 mM H₂DCFDA was added to each well, followed by incubation for 1 h at 37 °C. Subsequently, the solution was removed, wells washed with PBS, and plant extracts applied at the lowest and highest non-cytotoxic concentrations (mg/mL) for 1 h. Post-exposure, extract-containing media were discarded, wells washed twice with PBS, and 100 μ L of PBS added to each well. Medium was used as control; PBS was used as blank and 250 μ M H₂O₂ was used as positive control. All steps were performed on ice and in the dark, after which fluorescence was measured spectrofluorometrically (absorption: 485 nm, emission: 520 nm) in each well [21].

Genotoxicity determination

The single-cell gel electrophoresis (Comet) assay was employed to assess DNA damage induced by the plant extracts. The alkaline comet assay was performed under alkaline conditions (pH > 13) as previously described with minor modifications. Cells were cultured in appropriate plates, treated with extracts at the lowest and highest non-cytotoxic concentrations (in mg/mL) for 24 h, and then trypsinized

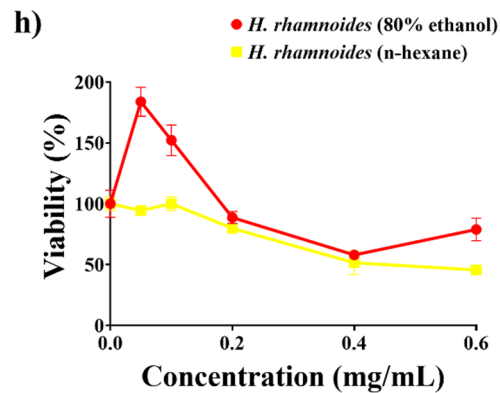
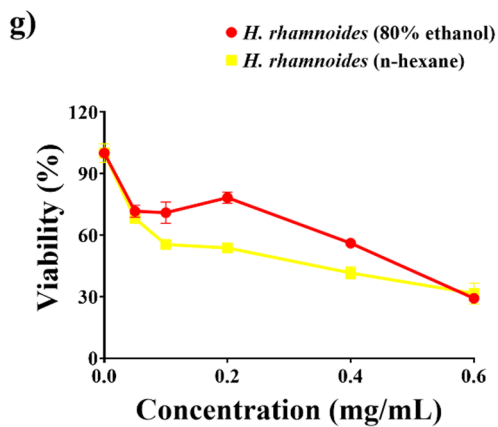
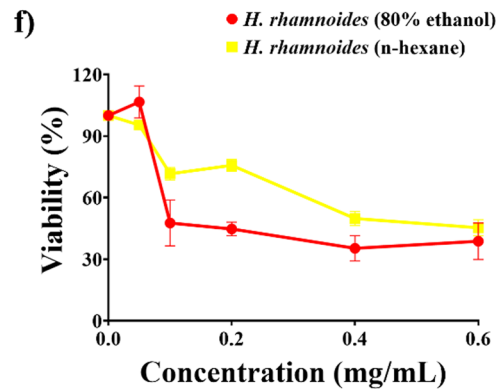
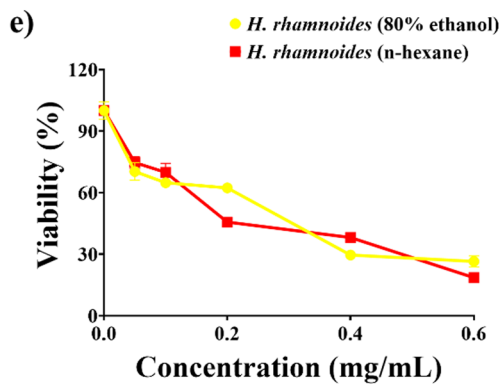
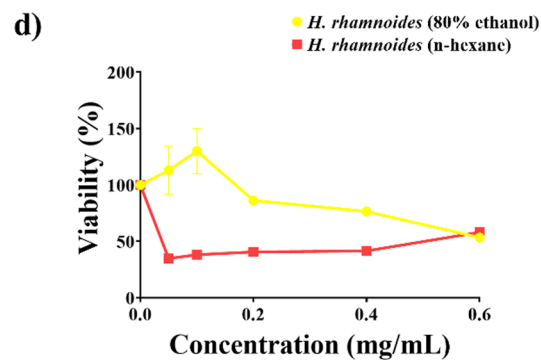
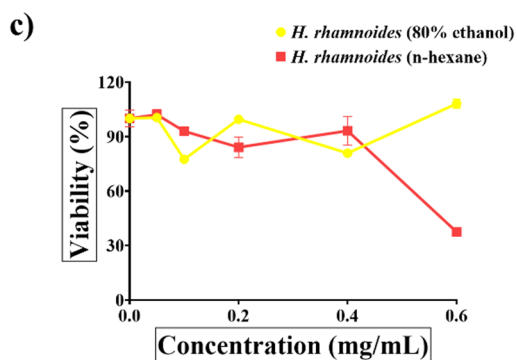
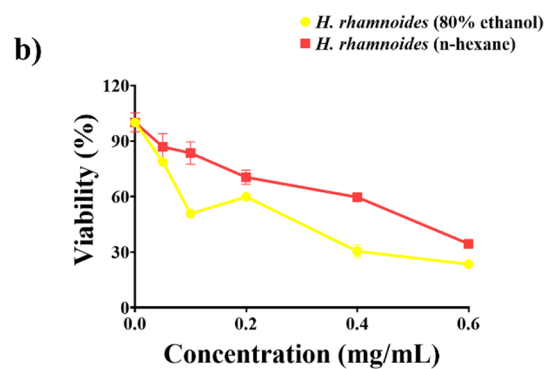
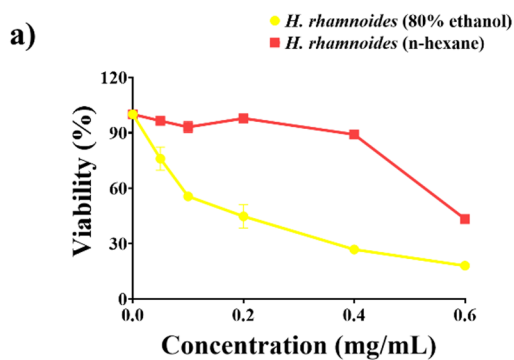


Table 1 IC₅₀ values of *H. rhamnoide*s extracts in breast cells

	IC ₅₀ (mg/mL)			
	MCF-7 ethanol	MCF-7 <i>n</i> -hexane	MCF-10 A ethanol	MCF-10A <i>n</i> -hexane
24 h	0,13	0,591	1,476	1,632
72 h	0,1	0,671	0,253	0,645

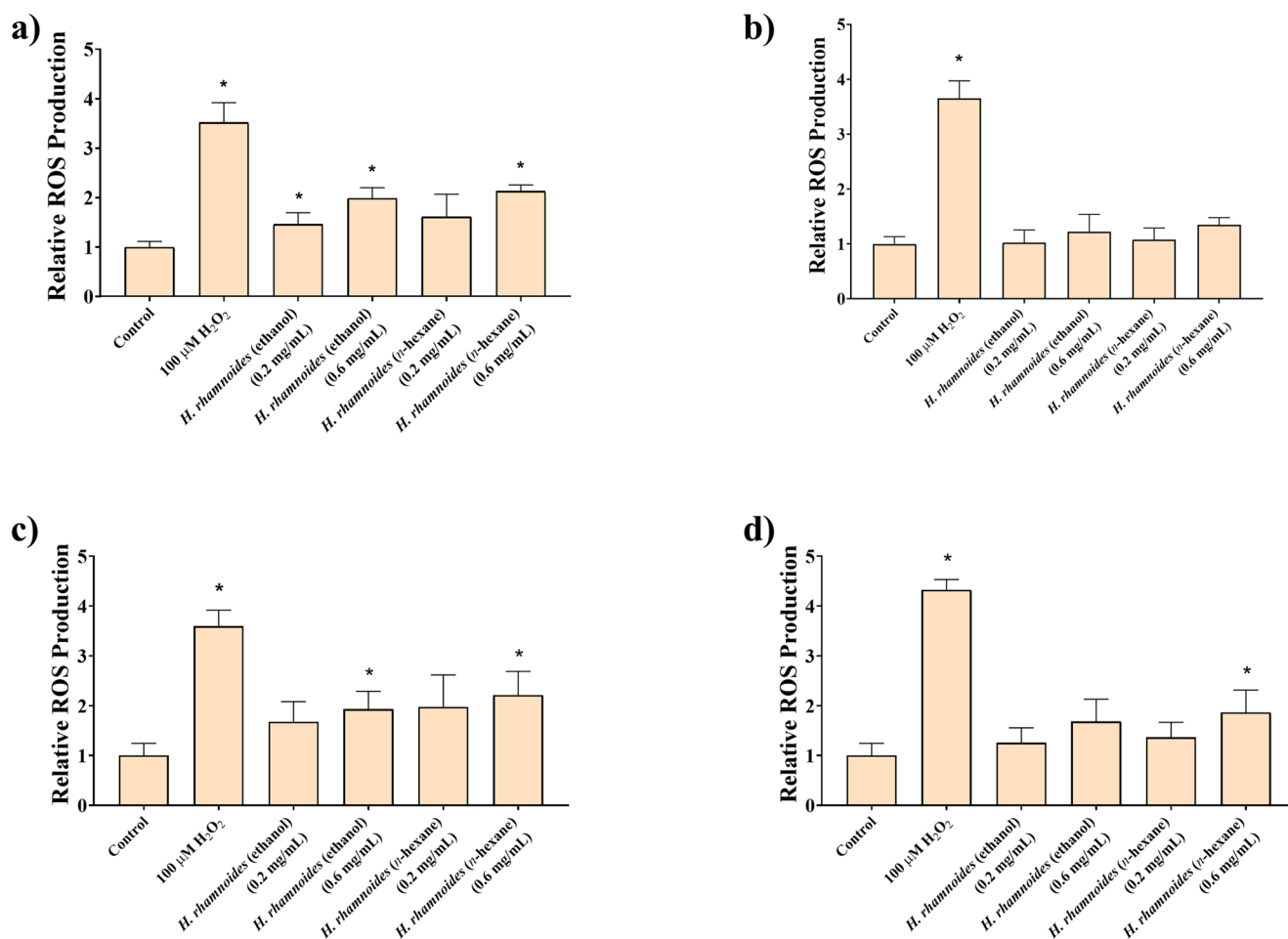
Table 2 IC₅₀ values of *H. rhamnoide*s extracts in colon cells

	IC ₅₀ (mg/mL)			
	HT-29 ethanol	HT-29 <i>n</i> -hexane	FHC ethanol	FHC <i>n</i> -hexane
24 h	0,18	0,155	0,590	0,365
72 h	0,076	0,242	0,142	0,423

Table 3 Selectivity index values of *H. rhamnoide*s extracts in breast and colon cells

	Breast cells		Colon cells	
	Ethanol	<i>n</i> -hexane	Ethanol	<i>n</i> -hexane
24 h	11,35	2,76	3,28	2,35
72 h	2,53	0,96	1,87	1,75

following PBS washing. The cell suspension was mixed with 1% low-melting-point agarose (LMA) and applied to slides precoated with 1% normal-melting-point agarose (NMA), which were allowed to solidify before lysis in lysis solution. The slides were immersed in freshly prepared cold lysis solution containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris-HCl, 1% Triton X-100, and 10% DMSO (pH 10) for at least 1 h at 4 °C. Subsequently, slides were incubated in alkaline electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH > 13) for 20 min to allow DNA unwinding and expression of alkali-labile sites. Electrophoresis was then conducted at 25 V and approximately 300 mA for 20 min under cold conditions. Post-electrophoresis, slides were neutralized in PBS for 15 min, dehydrated stepwise in 50%, 75%, and 99% ethanol (5 min each), stained with EtBr, and analyzed for tail intensity (%) under a fluorescence microscope (40× magnification) using Perceptive Instruments Comet IV software (100 cells per slide). Media containing 1% DMSO served as the negative control [21, 23].

**Fig. 2** Relative ROS production in (a) MCF-7, (b) MCF-10 A, (c) HT-29, (d) FHC cell lines following treatment with *H. rhamnoide*s ethanolic and *n*-hexane extracts. * compared with the negative control, $p < 0.05$. Data were expressed as mean ± standard deviation

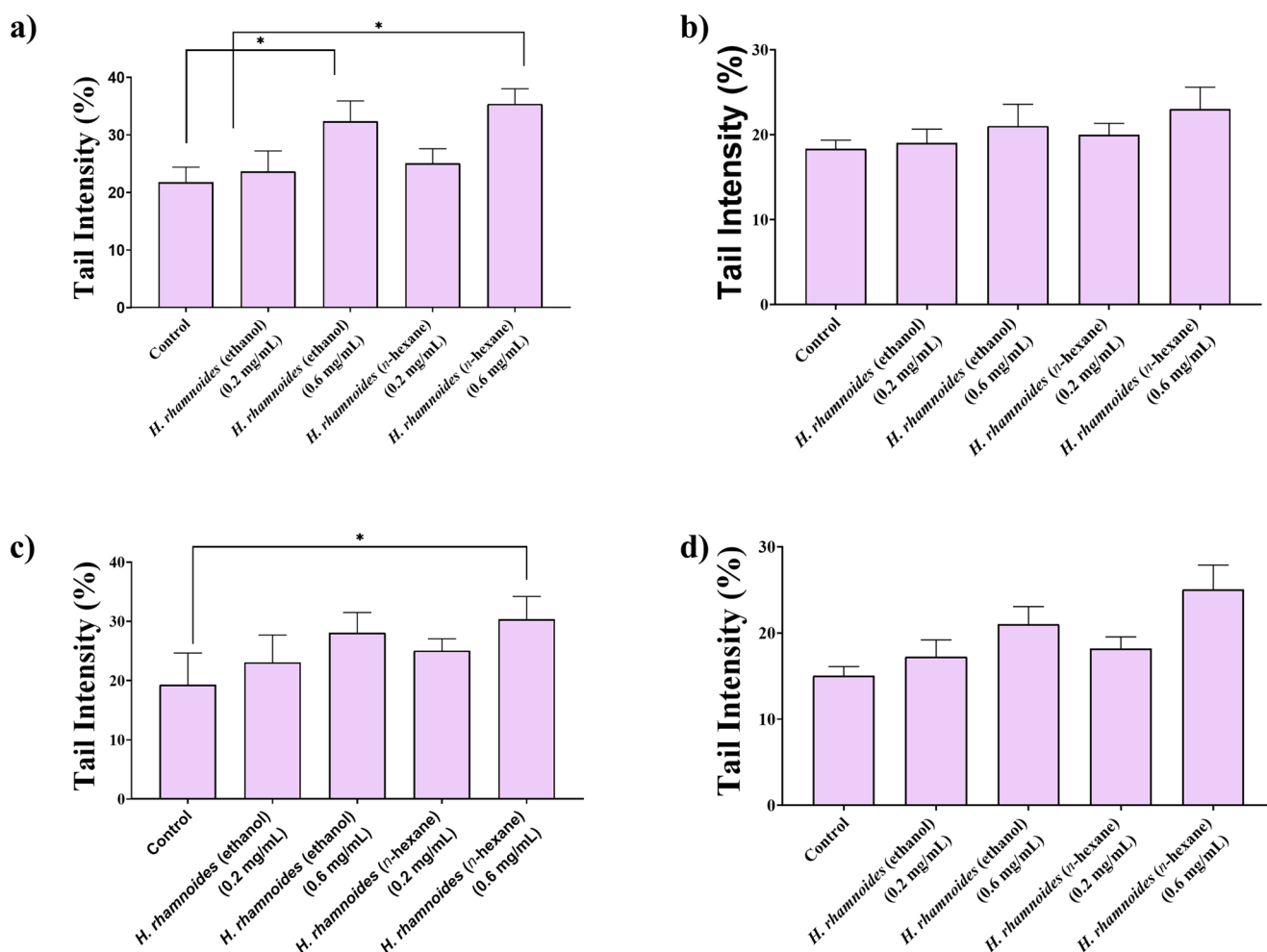


Fig. 3 Genotoxicity assessment of *H. rhamnoides* ethanolic and n-hexane extracts in (a) MCF-7, (b) MCF-10 A, (c) HT-29, (d) FHC cell lines after 24 h exposure; * compared to negative control ($p < 0.05$); ** compared between groups ($p < 0.05$). Data were expressed as mean $\pm$ standard deviation

Apoptosis analysis

Cells were cultured as described in the Cell Culture section, seeded at a density of 5×10^5 cells/well in 6-well plates, and incubated at 37 °C for 24 h. Following incubation, the media were discarded, wells were washed with PBS, and cells were treated with plant extracts at the lowest and highest non-cytotoxic concentrations (mg/mL) for 24 h. Post-treatment, the media containing extracts were removed, wells washed with PBS, and cells detached by trypsinization. Apoptosis was then assessed using an Annexin V–propidium iodide (PI) kit according to the manufacturer's instructions, followed by flow cytometric analysis (CytoFLEX, Beckman Coulter, USA). Using dot-plot gating, four distinct cell populations were identified: viable cells, early apoptotic cells, late apoptotic cells, and necrotic cells. Data were analyzed via quadrant-based categorization to quantify the percentage distribution of each population. Compensation settings were established using single-stained controls

prior to sample acquisition. During analysis, cellular debris and doublets were excluded based on forward and side scatter characteristics before quadrant gating. Apoptotic cell populations were quantified according to Annexin V-FITC/PI staining profiles, including viable, early apoptotic, late apoptotic, and necrotic cells. A minimum of 10,000 events were acquired for each sample [21].

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High performance liquid chromatography (HPLC) analysis

For HPLC analysis, the ethanolic extract obtained from *H. rhamnoides* fruits was dissolved in 80% acetonitrile (ACN) at a concentration of 10 mg/mL and passed through a 0.45 μ m PTFE membrane filter prior to injection. All analyses were carried out in three replicates on an Agilent 1260 series liquid chromatography system (Agilent, GL Sciences Inc., Waldbronn, Germany), equipped with a quaternary pump (G1311B), a diode array detector (G7115A), and a system controller.

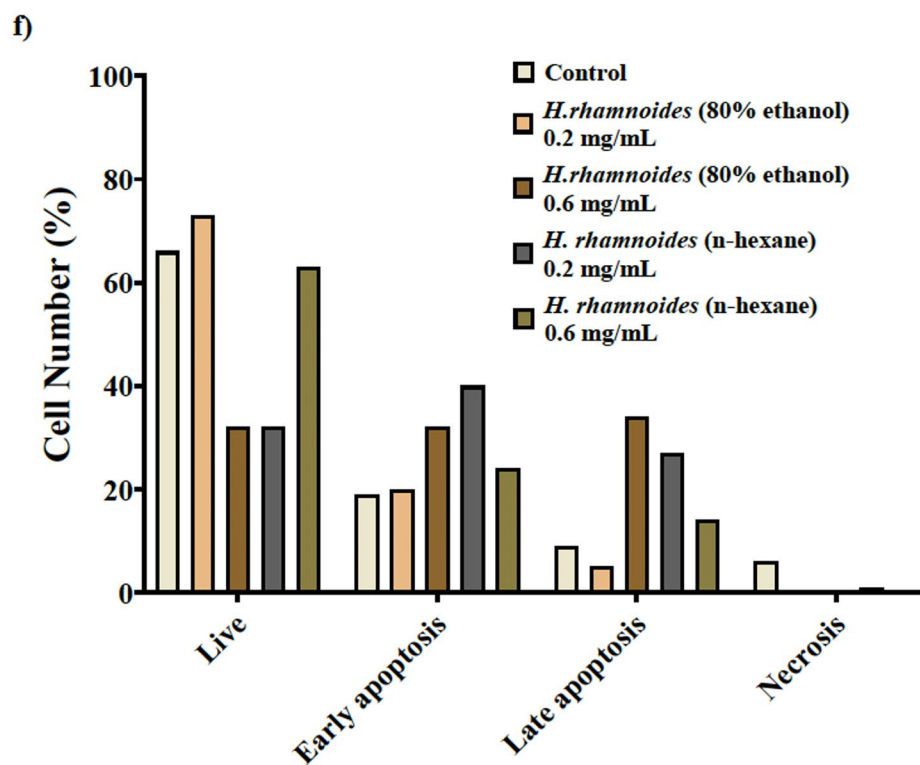
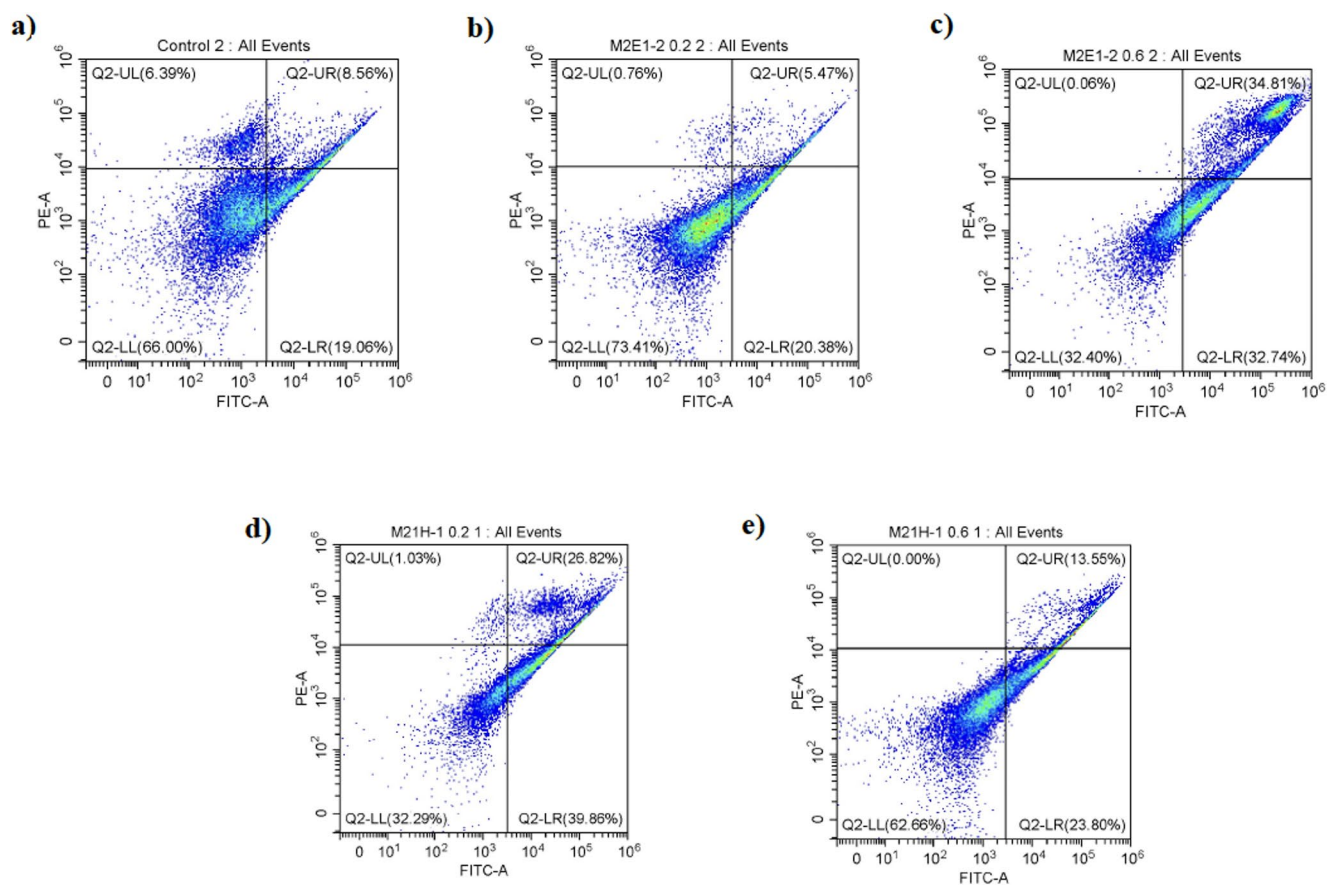


Fig. 4 Flow cytometric analysis of apoptosis in MCF-7 cells. Cells stained with Annexin V-FITC and PI. **(a)** Negative control (1% DMSO), **(b)** *H. rhamnoides* ethanolic extract (0.2 mg/mL), **(c)** *H. rhamnoides* ethanolic extract (0.6 mg/mL), **(d)** *H. rhamnoides* n-hexane extract (0.2 mg/mL), **(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

Separation of the analytes was achieved on an ACE 5 C18 reversed-phase column (150 mm × 4.6 mm, 5 µm particle size) held at a constant temperature of 25 °C. The injection volume was set to 20 µL. A binary mobile phase was employed, consisting of a mixture of acetonitrile and ultrapure water (80:20, v/v) containing 0.1% formic acid (Solvent A) and ultrapure water containing 0.1% formic acid (Solvent B), run under a gradient elution program adapted from Ekin et al. (2019) with slight modifications [24].

The gradient elution program was as follows: initial conditions were set to 5% A / 95% B at a flow rate of 0.800 mL/min, linearly increased to 15% A / 85% B at 10 min, held until 15 min. The flow rate was then reduced to 0.600 mL/min and held until 20 min, after which it was restored to 0.800 mL/min. The mobile phase composition was then increased to 20% A / 80% B at 24 min, 30% A / 70% B at 34 min, and 100% A / 0% B at 52 min, where the flow rate was increased to 1.000 mL/min and held until 55 min. The total run time was 55 min, followed by a 5-minute re-equilibration to initial conditions.

The analytical method was applied to screen a broad spectrum of phenolic acids and flavonoids. The phenolic acids investigated included gallic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, ellagic acid, fumaric acid, rosmarinic acid, quinic acid, protocatechuic acid, protocatechuic aldehyde, and p-hydroxybenzoic acid. Flavonoid compounds such as catechin, epicatechin, epigallocatechin, rutin, luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, quercetin-3-*O*-glucoside, hesperidin, myricetin, quercetin, luteolin, apigenin, kaempferol, and naringenin were also targeted. Quantitative determination of the identified compounds was carried out by constructing calibration curves at six different concentration levels, based on the relationship between peak area and concentration.

Among all the screened compounds, four phenolic constituents were successfully identified in the extract: protocatechuic acid, rutin, quercetin, and quercetin-3-*O*-glucoside. The concentrations of these detected compounds were quantitatively determined using the corresponding calibration curves, and their respective contents in the extract were calculated accordingly.

Statistical analysis

Data were analyzed using SPSS version 20.0 (SPSS Inc., Chicago, IL). Normality was assessed via the Kolmogorov–Smirnov test, and homogeneity of variance was assessed using Levene’s test. Statistical differences between groups were evaluated using Student’s t-test or ANOVA followed by LSD post hoc test. A p-value < 0.05 was considered statistically significant. All experiments were conducted independently three times with duplicate samples.

Results and discussion

Cytotoxicity

The effects of *H. rhamnoides* extracts with different polarities on breast and colon cells were evaluated in short-term (24 h) and long-term (72 h) exposure. The extracts generally exhibited lower cytotoxicity in non-tumorigenic cells than in cancer cell lines, although reductions in viability were observed under certain exposure conditions, particularly at higher concentrations and longer incubation periods. However, a decrease in cell viability was detected with increasing extract concentration in both cancer types. Furthermore, it is noteworthy that the n-hexane extract increased cancer cell proliferation, especially in colon cancer cells, with long-term exposure (Fig. 1). Compared with the n-hexane extract, the ethanolic extract demonstrated a comparatively more favorable cytotoxicity profile under the experimental conditions employed. This selectivity, likely driven by the varying phenolic and flavonoid compositions inherent to the polarity of the solvents, suggests a higher therapeutic window for the more polar phytochemical profiles. Also, the IC₅₀ values were given in Table 1 (breast cells) and Table 2 (colon cells).

Selectivity index calculation

The selectivity index was calculated as the ratio of the IC₅₀ value for the healthy cell line to that of the corresponding cancer cell line. Higher SI values indicate a wider therapeutic window, comparatively lower cytotoxicity toward non-tumorigenic epithelial cells [25]. As shown in Table 3, all extracts -except the n-hexane fraction on breast cells at 72 h- demonstrated selective cytotoxicity against cancer cells across all exposure durations. The cytotoxic effects of the extracts in non-malignant cells were dependent on extract polarity, cell type, and exposure duration. Although the ethanolic extract exhibited a more favorable selectivity profile under several

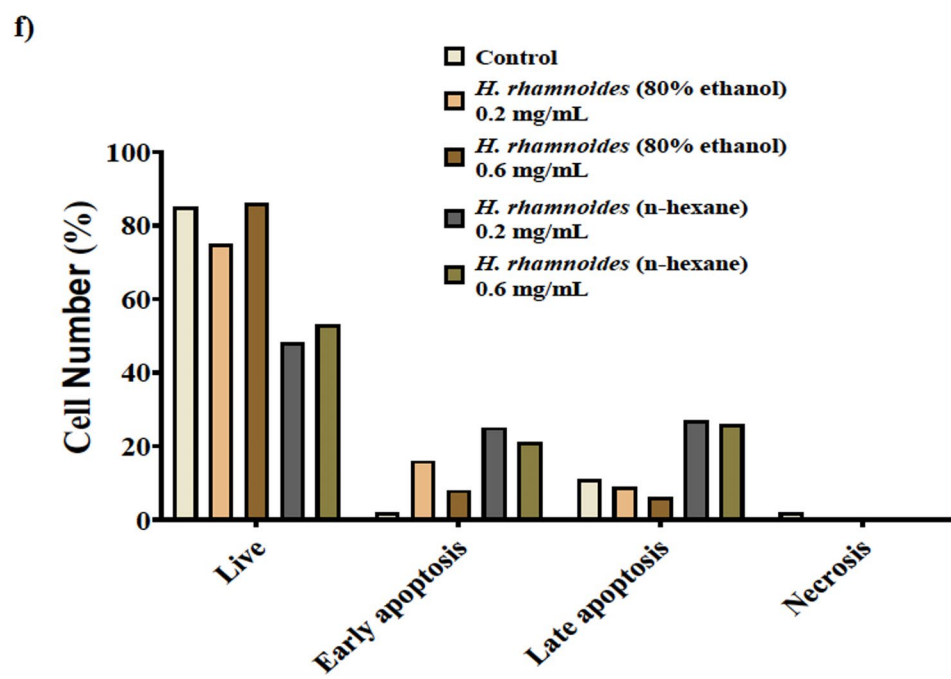
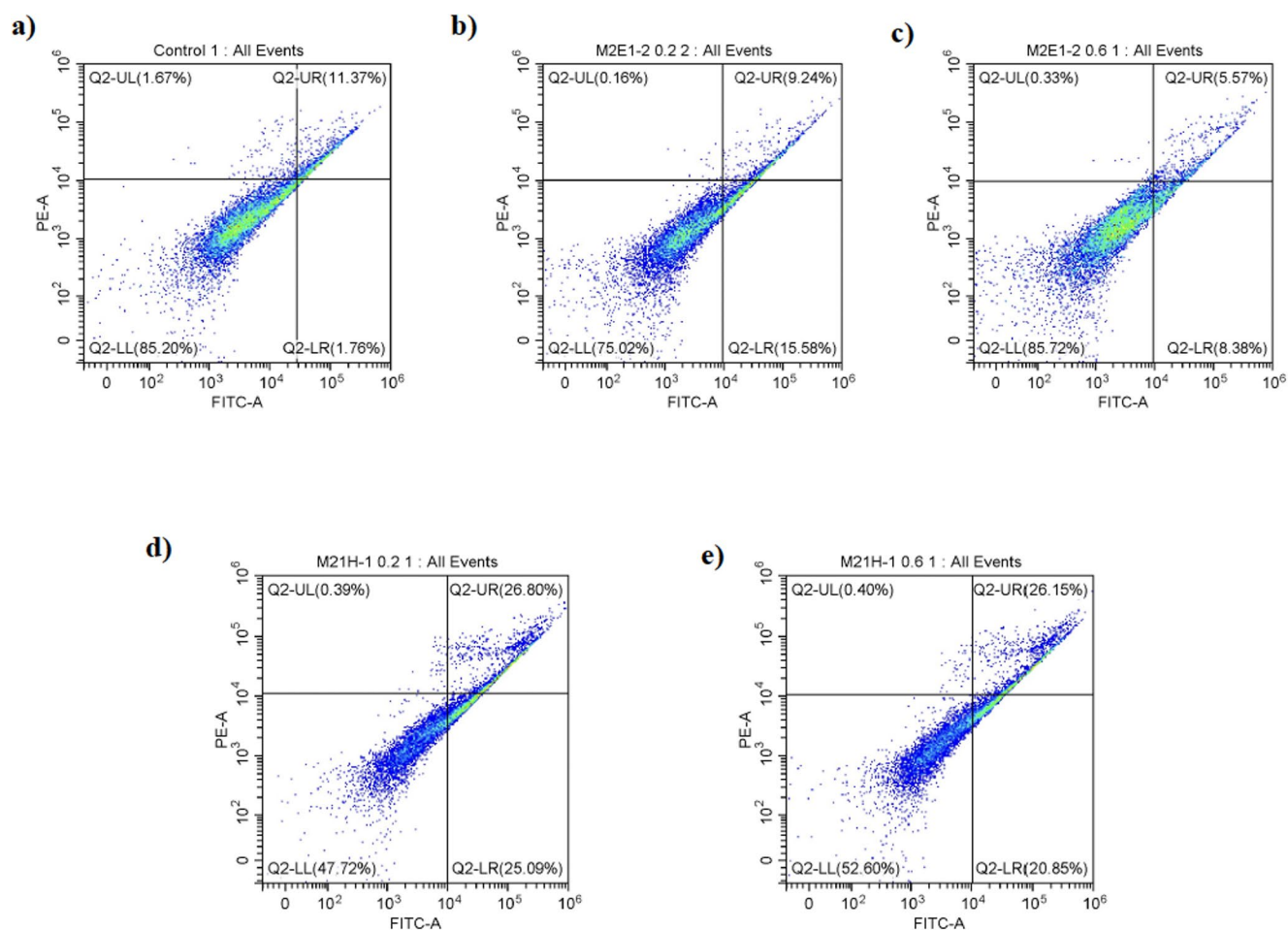


Fig. 5 Flow cytometric analysis of apoptosis in HT-29 cells. Cells stained with Annexin V-FITC and PI. **(a)** Negative control (1% DMSO), **(b)** *H. rhamnoides* ethanolic extract (0.2 mg/mL), **(c)** *H. rhamnoides* ethanolic extract (0.6 mg/mL), **(d)** *H. rhamnoides* n-hexane extract (0.2 mg/mL),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

experimental conditions, higher IC₅₀ values in non-malignant cells were not consistently observed across all treatment durations [26]. These findings align with prior studies highlighting high SI values as indicators of therapeutic potential in oncology [27] and emphasize the value of polarity-based fractionation for isolating compounds that preferentially disrupt cancer-specific metabolic pathways [28].

While *H. rhamnoides* extracts demonstrated a general tendency toward higher cytotoxicity in cancer cell lines compared to non-tumorigenic counterparts, this effect was not uniformly selective across all conditions. In particular, n-hexane extracts induced a measurable reduction in viability in healthy cells, and selectivity index values approached or fell below unity at certain time points (e.g., SI=0.96 at 72 h in breast cells), indicating limited or absent selectivity under these conditions. These findings suggest that the cytotoxic effects of the extracts are influenced by solvent polarity, concentration, and exposure duration, and that selective anticancer activity is context-dependent rather than universal.

ROS production

Phytochemicals derived from plants are well-established for their antioxidant properties [29]. Nevertheless, these extracts display a complex redox-modulating capacity and may promote oxidative stress under certain cellular and experimental conditions, particularly in malignant cells [30]. ROS production increased in a cell type- and concentration-dependent manner following treatment with *H. rhamnoides* extracts. In MCF-7 cells, the positive control induced approximately a 3.5-fold increase in ROS levels, while the ethanolic and n-hexane extracts increased ROS production by approximately 1.5- to 2.1-fold relative to the control ($p < 0.05$ for significant groups). Similar increases were observed in HT-29 cells, reaching approximately 1.7- to 2.2-fold of control values. In non-tumorigenic cells, ROS responses were generally less pronounced; however, the higher concentration of the n-hexane extract increased ROS levels in FHC cells to approximately 1.9-fold of the control value ($p < 0.05$) (Fig. 2). These findings suggest that oxidative stress may contribute to the biological effects of the extracts,

although the magnitude of the response was dependent on cell type and exposure conditions.

Genotoxicity determination

The genotoxicity results revealed differential DNA damage responses between malignant and non-tumorigenic cell lines; however, the magnitude and direction of these effects varied according to extract polarity and treatment conditions. In healthy cell models, polar extracts did not reduce basal DNA damage below control levels, suggesting that a protective effect cannot be conclusively inferred under the tested conditions. Overall, these findings indicate a differential, but not absolute, genotoxic response, highlighting that the effects of the extracts are context-dependent rather than strictly cancer-specific (Fig. 3).

Apoptosis analysis

The apoptosis analysis revealed treatment-dependent changes in Annexin V+/PI⁺ and Annexin V+/PI⁻ cell populations, indicating that the extracts were capable of modulating apoptotic responses under certain experimental conditions. 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 [31].

Apoptosis analysis revealed a dose-dependent but non-linear response to n-hexane extracts. In MCF-7 cells, the lower concentration induced a more pronounced apoptotic effect compared to the higher concentration, suggesting that increasing dose does not necessarily correspond to increased apoptosis. This may reflect a shift toward alternative cell death mechanisms, such as necrosis, at higher concentrations. In contrast, HT-29 cells exhibited a more consistent increase in apoptosis with increasing concentration. These findings indicate that the apoptotic response is both dose- and cell type-dependent, rather than uniformly increasing with concentration (Figs. 4 and 5).

HPLC analysis

Based on the selective cytotoxic activity observed for the polar extracts, HPLC analysis was performed exclusively on the ethanolic extract of *H. rhamnoides* fruits to identify phytochemical constituents potentially associated with this activity. Among all the screened compounds, four phenolic constituents were successfully identified and quantified: protocatechuic acid, rutin, quercetin-3-*O*-glucoside, and quercetin. All compounds were quantified over a calibration

Table 4 HPLC quantification of phenolic compounds identified in the ethanolic extract of *H. rhamnoides* fruits: retention times, detection wavelengths, calibration parameters, and their concentrations in the extract

Compounds	Range (ppm)	Retention time(min)	The absorbance of detection (nm)	Standard curve	R^2	Amount in the extracts (mg/g) (Mean $\pm$ S.D.)
Protocatechuic acid	1–125	9,618	260	$y = 145,29x - 38,724$	0,9998	$0,4906 \pm 0,0049$
Rutin	1–125	31,429	260	$y = 42,784x - 18,281$	0,9998	$3,3517 \pm 0,0317$
Quercetin 3- <i>O</i> -glucoside	1–125	32,361	260	$y = 63,577x - 26,534$	0,9998	$0,1897 \pm 0,0030$
Quercetin	1–125	40,947	260	$y = 67,016x - 15,029$	0,9999	$0,3026 \pm 0,0081$

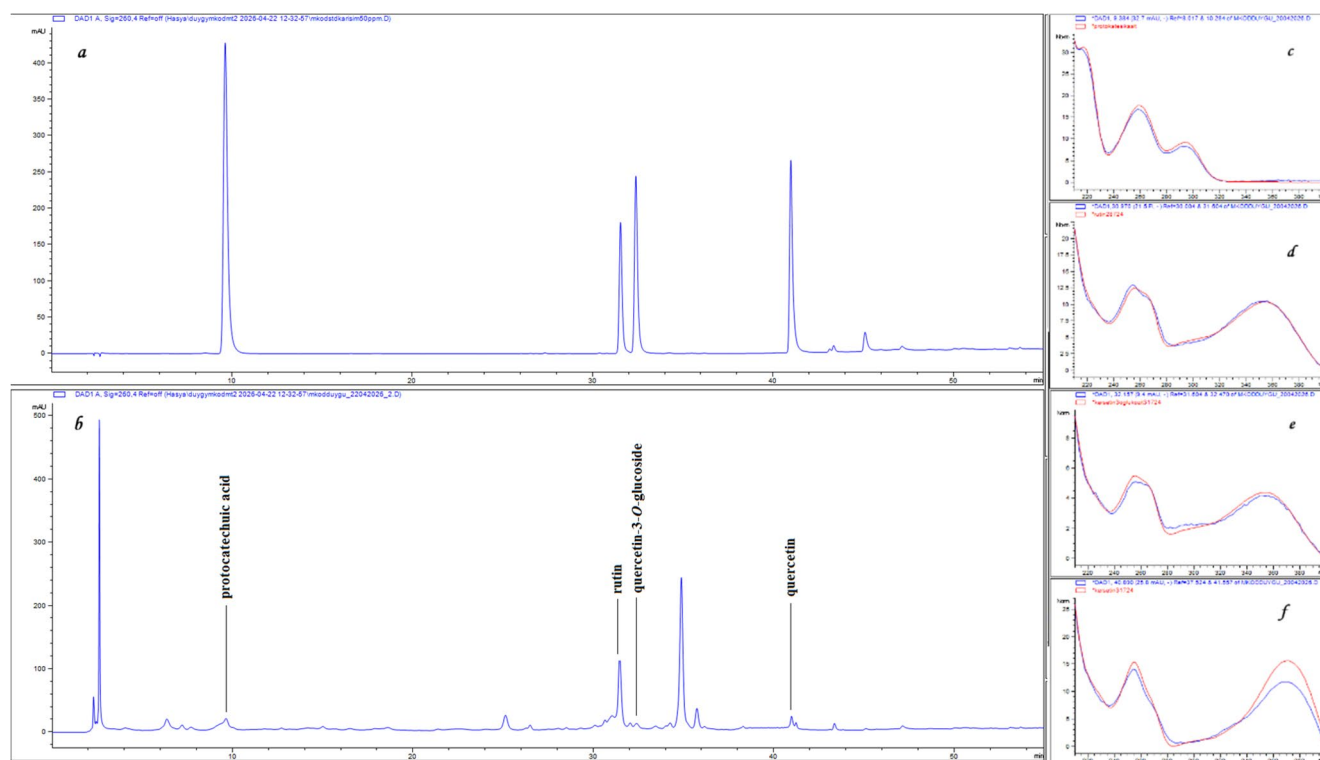
range of 1–125 ppm at 260 nm, and all calibration curves demonstrated excellent linearity, with R^2 values of 0.9998–0.9999, confirming the reliability of the quantification method. The quantification results are presented in Table 4, and the representative HPLC chromatogram of the extract is illustrated in Fig. 6.

Rutin was found to be the predominant compound in the extract, with a content of 3.3517 ± 0.0317 mg/g, followed by protocatechuic acid at 0.4906 ± 0.0049 mg/g. Quercetin was detected at a concentration of 0.3026 ± 0.0081 mg/g, while quercetin-3-*O*-glucoside was present at the lowest level among the identified compounds, with a content of 0.1897 ± 0.0030 mg/g. The chromatographic separation of these compounds was achieved at retention times of 9.618, 31.429, 32.361, and 40.947 min, respectively, indicating good resolution across the analytical run.

Considering the previously reported antioxidant, antiproliferative, and pro-apoptotic activities of these phenolic compounds and flavonoid derivatives (rutin, quercetin, protocatechuic acid, and quercetin-3-*O*-glucoside), their presence in the bioactive ethanolic extract suggests that they may be among the constituents contributing to the observed activity [32–41]. However, the potential contribution of other unidentified or unquantified constituents present in the extract should also be taken into consideration.

Conclusion

The prevention and treatment of chronic diseases, particularly cancer, through dietary phytochemicals, are gaining increasing importance.

**Fig. 6** HPLC analysis of phenolic compounds in the ethanolic extract of *H. rhamnoides* fruits at 260 nm. (a) standard mixture chromatogram; (b) extract chromatogram; (c–f) UV spectral overlays of peaks

in the extract with their corresponding standards at same Rt: (c) protocatechuic acid, (d) rutin, (e) quercetin-3-*O*-glucoside, (f) quercetin

This study demonstrates that *Hippophae rhamnoides* fruit extracts exhibit a clear polarity-dependent cytotoxic profile. Notably, polar extracts show enhanced selectivity toward cancer cells, primarily through targeted modulation of oxidative stress and induction of apoptosis. The differential regulation of ROS further supports the ability of these phytochemical fractions to disrupt cancer-specific redox homeostasis.

Importantly, these findings highlight the critical role of solvent polarity in shaping the bioactivity and selectivity of phytochemical extracts. This polarity-driven selectivity represents a key novelty of the study and suggests that optimized extraction strategies may improve the therapeutic potential of diet-derived compounds. From a safety perspective, the induction of DNA damage should be interpreted with caution. While DNA damage in cancer cells may contribute to cytotoxic or antiproliferative effects, genotoxic responses in non-tumorigenic cells may indicate a potential safety concern, particularly for dietary bioactive compounds. Therefore, the observed comet assay results should not be considered solely as evidence of anticancer efficacy, but also as an indication that further genotoxicity and safety evaluations are required. Further in vitro and in vivo investigations are warranted to validate these effects and facilitate their clinical applicability.

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

Declarations

Ethical approval Ethics approval was not required for this research.

Conflict of interest The authors declare there are no conflicts of interest.

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