BMC Complementary Medicine and Therapies

RESEARCH

Open Access

Trachystemon orientalis (L.) G. Don aqueous extract induces cell cycle arrest and apoptosis in MCF-7 breast cancer cells via multi-pathway modulation

Yagmur Demirel Ozbek[1*], Levent Gulum[2], Ayse Busranur Celik[3,4], Ozlem Saral[1], Yigit Eren Yıldız[5], Lütfi Tutar[6] and Yusuf Tutar[4,7]

Abstract

Background/objectives *Trachystemon orientalis* (TO) is native to Bulgaria, the western Caucasus, and Turkey. It possesses antimicrobial, antifungal, and antidiabetic properties. Due to their efficacy and lower toxicity, the search for natural compounds with anticancer potential, especially for breast cancer, has increased. This study evaluated the cytotoxic effects on breast cancer cells.

Methods MCF-7 breast cancer cells and CCD-1072Sk normal fibroblast cells were treated with TO extracts prepared using water, methanol, ethanol, and dimethyl sulfoxide (DMSO). Cytotoxicity was assessed in a dose- and time-dependent manner, and IC_{50} values were calculated. The most potent extract, water, was analyzed for its effects on cancer-related pathways via RT-qPCR, gene enrichment, and gene-metabolite association analyses. Flow cytometry was used to assess cell cycle distribution and apoptosis, and fluorescence staining was used to evaluate nuclear, cytoskeletal, and mitochondrial morphology.

Results TO exhibited dose-dependent cytotoxicity in MCF-7 cells, with IC_{50} values ranging from 28.88 ± 1.91 ng/µL (water extract) to 185.30 ± 6.60 ng/µL (ethanol extract), demonstrating lower toxicity in normal cells. KEGG pathway enrichment analysis showed that the water extract was associated with the modulation of several cancer-related signaling pathways, including p53, HIF-1, TNF, PI3K–Akt, and MAPK pathways, which induced S-phase arrest and increased apoptosis by 11% as confirmed by morphological changes.

Conclusions Taken together, these findings indicate that TO has cytotoxic and apoptosis-inducing effects on breast cancer cells through the regulation of multiple signaling pathways and is a potential candidate for further validation in breast cancer research.

Keywords MCF-7 cell line, *Trachystemon orientalis* (L.) G. Don, Molecular mechanism, Anticancer activity

*Correspondence:
Yagmur Demirel Ozbek
yagmur.demirel@erdogan.edu.tr

Full list of author information is available at the end of the article

Background

Cancer is defined as the excessive and uncontrolled division of cells [1]. Uncontrolled cell proliferation and the inability of cells to go to apoptosis are key contributors to cancer formation [2]. Disruptions in any apoptotic pathway lead to the formation, progression, and metastasis of cancer cells [3, 4]. Cancer remains one of the most important health problems among social and economic problems in the 21 st century. In fact, cancer is responsible for 22.8% of global deaths from non-communicable diseases (NCDs) [5]. According to a recent study, in 2020, nearly 1 million children lost a parent to cancer, with almost half of those children losing their mother to breast or cervical cancer [6]. In 2022, there were an estimated 20.0 million new cancer cases and 9.7 million cancer-related deaths worldwide. In these data, breast cancer was reported as the most prevalent cancer type among women [7]. It is predicted that there will be an increase in cancer cases in the coming years, especially in developing countries, due to factors such as rising environmental pollution, tobacco use, lifestyle changes, and malnutrition [8].

Breast cancer is the second leading cause of death among women. Breast cancer alone accounts for 31% of all cancer cases [9]. Although numerous steps have been taken in the treatment and diagnosis of breast cancer, its effectiveness is still not clearly proven, and treatment approaches are still inadequate. Today, breast cancer treatment protocols, including surgery, chemotherapy, radiotherapy, and immunotherapy have been established for breast cancer treatment, but it is reported that the obstacle to the methods used is that while destroying tumor cells, they also damage healthy cells [10]. While the development of tumor cells is supported by oncogenes, tumor suppressor genes are involved in preventing the formation of cancer cells by controlling processes such as cell cycle and cell death to prevent tumor formation at the molecular level [11, 12].

Plants are considered valuable and promising sources for developing new drugs against cancer, with reports indicating that approximately 60% of anticancer agents are derived from natural sources [13]. Anticancer compounds derived from plants have been observed to exhibit fewer side effects compared to chemical drugs [8, 14] and also suppress cellular proliferation and cell invasion by increasing apoptotic cell death. In addition to these benefits, it has also been proven that plant-derived compounds stimulate the immune system [10, 15, 16]. In addition, many plants are obscure and unproven in nature. Furthermore, it is reported that there is still a great need for research on new drugs derived from plants for the treatment of cancer [1, 2, 17].

Although *Trachystemon orientalis* (L.) G. Don (TO) is not a commonly known plant, it is widely consumed in Bulgaria, Western Caucasus, and Türkiye, especially in the Black Sea region [18]. This plant is rich in chlorophyll, β-carotene, lycopene, phenolics, free amino acids, glycine betaine, and ascorbic acid [19], making it an important source of antioxidants and phenolic compounds [20–22]. In previous studies on the TO plant, rosmarinic acid, rutin, myricetin, gallic acid, vanillic acid, caffeic acid, ferulic acid, hydrocinnamic acid, protocatechuic acid, and p-coumaric acid were detected, which have anticancer effects [18, 20–23]. The TO plant has significant antioxidant activity [18, 21] as well as antifungal [24], antidiabetic [25], anti-aging [26] and antimicrobial [27] effects in studies conducted.

There is limited current in vitro research on *Trachystemon orientalis* (TO) and cancer. According to a recent study, TO extract significantly slowed or inhibited the growth of brain cancer cells due to its phenolic compounds and antioxidant activity, demonstrating the potential of TO as a bioactive plant with cytotoxic and growth-inhibitory effects on cancer cells. However, further studies are required to clarify its molecular mechanisms and specific effects [28]. The present study introduces an integrative experimental framework combining IC_{50}-based post-treatment RT-qPCR, KEGG/GO enrichment analyses, cell cycle assessment, apoptosis evaluation, and gene–metabolite interaction analysis to explore the molecular responses induced by TO treatment.

Materials and methods

Chemicals

Solvents and all other chemical reagents were purchased from Merck and Sigma-Aldrich commercial suppliers. Dulbecco's Modified Eagle Medium High Glucose (DMEM) (EuroClone, Via Figino, Italy), fetal bovine serum (FBS) and MTT (3-(4,5-Dimethylthiazol-2-yl)–2,5-Diphenyl Tetrazolium Bromide) were purchased from Sigma-Aldrich (Darmstadt, Germany). ApopNexin Annexin-V-FITC with PI Apoptosis Kit was obtained from Merck (Darmstadt, Germany); Total RNA Isolation Kit was obtained from Analytik Jena (Jena, Germany); SensiFast cDNA Synthesis Kit was obtained from Meridian Bioscience (Cincinnati, USA); SybrGreen Master Mix was obtained from Euro-Clone (Via Figino, Italy).

Preparation of TO extract

TO was collected from Kurtuluş village (41.0351199, 40.5929884) in Rize, Türkiye, in March 2024. The plant material was taxonomically identified by Lütfi TUTAR and a voucher specimen was deposited in the Bozok University Herbarium (BOZOKHB), Department of Botany, under the accession number BOZOKHB0553. The plant leaves were collected by random sampling method, cleaned immediately after collection, and dried at room temperature (22–25 °C). The dried plant samples were

then ground into a fine powder using a blender (Waring, 8011ES).

The TO plant extract was prepared by modifying the method of Aksu and Derman. 2.5 ± 0.1 g of the dried plant was weighed and 50 ± 2 mL of solvent (methanol, distilled water, ethanol and DMSO) was added. Solvent diversity was provided by taking as an example the studies conducted previously with the plant TO [18–28]. All extracts were mixed with a mechanical shaker (Heidolph, MR3001) for 24 h at room temperature. After mixing, the suspensions were filtered through Whatman No. 1 filter paper to obtain clear extracts. Following filtration, the extracts were processed by applying minor modifications to the procedures previously used by the authors [29, 30]. The obtained crude extracts were stored at – 20 °C until use. Extract concentrations were adjusted appropriately prior to the experiments.

Cell culture and viability test

MCF-7, a human breast adenocarcinoma cell line (obtained from ATCC, Manassas, VA, USA), and CCD-1072Sk, a normal human skin fibroblast cell line (obtained from ATCC, Manassas, VA, USA), were used to investigate the cytotoxic effects of the active components of TO. Both cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM; Capricorn Scientific) supplemented with 10% fetal bovine serum (FBS; Euroclone) and 1% penicillin-streptomycin (Capricorn Scientific). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

The culture medium was replaced with fresh medium every 2–3 days, and the cells were used in the experiments when they reached 80–90% confluence. The effects of TO extracts on cell viability were evaluated using the MTT assay (Sigma-Aldrich (Darmstadt, Germany). MTT assay was conducted according to the manufacturer's instructions, and MCF-7 and CCD-1072Sk cells were seeded at 4.5×10^3 cells/well into 96-well plates and incubated for 24 h at 37 °C under 5% CO_2. TO extracts dissolved in four solvents (methanol, distilled water, ethanol, and DMSO) at eight doses (1200, 600, 300, 150, 75, 37.5, 18.75, and 9.375 µg/mL) were applied. After the extract application, cells were incubated for 72 h, a treatment duration commonly used in plant extract–based cytotoxicity studies and previously shown by our research group to yield consistent biological effects [31]. Following this incubation period, 10 µL of MTT solution (5 mg/mL) was added to each well and further incubated at 37 °C under 5% CO_2 for 4 h. Then, 100 µL of DMSO was added to each well to dissolve the purple formazan crystals, and the plates were incubated at room temperature with gentle shaking in the dark for 15 min. The optical density was measured using a microplate reader at a wavelength of 590 nm to determine the MTT reaction in the cells. All experiments were repeated in three replicates.

The selectivity index (SI) was calculated to evaluate the differential cytotoxicity of TO extracts between cancerous (MCF-7) and normal (CCD-1072Sk) cells. SI values were determined by dividing the IC_{50} value for normal cells by the IC_{50} value for cancer cells [32].

RT-qPCR and gene enrichment analysis

RT-qPCR was performed in triplicates to quantitatively analyze the external and internal apoptosis signaling pathways. MCF-7 cancer cells were seeded at 4.5×10^5 cells per well in 6-well plates for RNA isolation. After 24 h of incubation, water-soluble TO extract was applied with IC50 value. Only water was employed as a negative control group. After 72 h, total RNA was isolated using InnuPREP RNA Mini Kit 2.0 (Innuscreen GmbH) according to the manufacturer's instructions. The purity and concentration of the isolated RNA was determined using a NanoDrop spectrophotometer (BioSpec-nano Spectrophotometer, Shimadzu, USA). cDNA synthesis was performed using the Wonder RT-cDNA Synthesis Kit (Euroclone, Milan, Italy) as per the manufacturer's guidelines. The PCR cycle was set as 10 min at 25 °C, 15 min at 42 °C, and 5 minutes at 85 °C. The SYBR Green Master Mix (EuroClone) kit and Analitik Jena qTOWER3 RT-qPCR device were used for the RT-qPCR reaction. Gene-specific primers were custom-designed and commercially synthesized by Merck (Sigma-Aldrich) for quantitative real-time PCR analysis. The list of primers used in this study is provided in Supplementary Table 1. The thermal cycling conditions consisted of an initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. All samples were analyzed in duplicate. Gene expression levels were quantified using the 2^−ΔΔCt method [33], with GAPDH and ACTINB serving as reference genes for normalization. Genes with significant changes in expression (≥ 2-fold increase or ≤ 0.5-fold decrease) were identified and subjected to KEGG pathway analysis using the ShinyGO platform [34].

Gene-metabolite interaction analysis

Genes showing significant changes in expression (≥ 2-fold increase or ≤ 0.5-fold decrease) were selected for gene-metabolite analysis. Using the Enrichr platform [35–37], the "Metabolomics Workbench Metabolites" database was utilized to identify metabolites associated with these genes. Subsequently, the identified metabolites and genes were used to construct a gene-metabolite interaction network via the MetaboAnalyst 6.0 software [38].

Cell cycle analysis

Cell cycle analysis was performed using the Sigma-Aldrich MAK344 Cell Cycle Analysis Kit. The protocol steps provided by the manufacturer were followed. MCF-7 cells were cultured at a concentration of 3×10^5 cells/well and treated at the IC50 values determined for the sample for 72 h. At the end of the treatment period, the cells were washed with 500 µL of phosphate-buffered saline (PBS), trypsinized, and the cell pellets were collected by centrifugation at 400 rpm for 5 minutes. The collected pellets were washed with 2 mL of 1X cell cycle assay buffer and centrifuged again at 400 rpm for 5 minutes. The supernatant was discarded, and the cells were fixed by carefully adding 2 mL of cold 70% ethanol to the pellets slowly. The ethanol-treated cells were placed on ice for a minimum of 30 min. Then centrifugation was performed again, and the super-natant was discarded. In the next step, the pellets were washed again with 2 mL 1X cell cycle assay buffer and centrifuged again, and the supernatant was carefully discarded without cell loss. Next, 500 µL of staining solution, prepared with 100 µL of Enzyme A solution and 400 µL of nuclear dye in 1X buffer solution, was added to the cell pellets. The cells were incubated for 30 min at room temperature in a dark environment. After the incubation period, the cells were analyzed using flow cytometry to assess their cell cycle distribution.

Apoptosis analysis

The FITC Annexin-V apoptosis Detection Kit with PI, ApopNexinTM FITC (APT750, Merk, Germany) was used for apoptosis detection. Total MCF-7 cells were seeded in 6-well plates at a concentration of 1×10^5 cells/well. MCF-7 cells were cultured with a cytotoxic concentration (IC_{50}) of mg/ml for a duration of 72 h. After incubation with the extract for 72 h, the medium was removed and the cells were washed with phosphate-buffered saline (PBS) and centrifuged at 400 rpm for 5 min after removal with Trypsin-EDTA. The cells were then washed twice with 5 mL of pre-cooled PBS.

The cells were then resuspended in 1 mL of cold 1X Binding Buffer (Sigma Aldrich, USA). From this suspension, 200 µL the mixture was transferred to a flow cytometry tube, while the remaining cells were kept on ice. Subsequently, 3 µL of ApopNexin™ FITC and 2 µL of 100X propidium iodide (PI) (P4170, Sigma Aldrich, USA) were added to the flow cytometry tube and mixed thoroughly. The mixture was incubated at room temperature in the dark for 15 min. At the end of the incubation period, the samples were analyzed using flow cytometry, and data were collected for apoptosis detection.

Cell morphology analysis

MCF-7 cells were seeded into 96-well plates at a density of 3×10^3 cells per well. After 24 h of incubation, the medium was replaced with a medium containing the extract at its IC50 value. Staining was performed after 72 h of treatment. Nuclei (Hoechst 33342, Sigma Aldrich), mitochondria (BioTracker 633 Red Mitochondria Dye, Sigma Aldrich), and cytoskeleton (BioTracker 488 Green Microtubule Cytoskeleton Dye, Sigma Aldrich) staining were performed according to the manufacturer's instructions. Briefly, after removing the culture medium from the wells, the cytoskeleton dye was prepared at a 1X concentration in fresh medium and applied to the wells, followed by a 15-minute incubation. Subsequently, a nuclear dye at 1 µg/mL and a mitochondrial dye at 200 nM were added to the wells and incubated together for an additional 15 min. Following incubation, the staining solution was discarded, fresh medium was added, and morphological changes were assessed using the ZOE fluorescence microscope.

Statistical analysis

Viability data were analyzed using GraphPad Prism version 8.0.2. Cell viability was calculated as the percentage of absorbance relative to the untreated control group, which was set to 100%. Absorbance values were normalized to controls and used to generate dose–response curves. The half-maximal inhibitory concentration (IC_{50}) values were calculated by nonlinear regression using a sigmoidal dose–response (variable slope) model.

Data are presented as mean ± standard deviation (SD) from two independent experiments, each performed in triplicate. Statistical significance was assessed using one-way and two-way ANOVA as appropriate. For comparisons between two groups, an unpaired Student's t-test was applied. Gene expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method. Flow cytometry data were analyzed using CytExpert software. A p-value < 0.05 was considered statistically significant.

Results

MTT analysis results

In this study, extracts of TO were prepared using four different solvents (DMSO, water, methanol, and ethanol). The anti-proliferative activity of these extracts on MCF-7 cancer cells was assessed using the MTT assay. The inhibition effects of the extracts at various concentrations (1200, 600, 300, 150, 75, 37.5, 18.75, and 9.375 µg/mL) on MCF-7 cancer cells were evaluated after 72 h of treatment (Fig. 1).

Comparative analysis of the plant extracts revealed that the water extract demonstrated the most potent anti-proliferative activity, while the ethanol extract exhibited the least efficacy against MCF-7 breast cancer cells

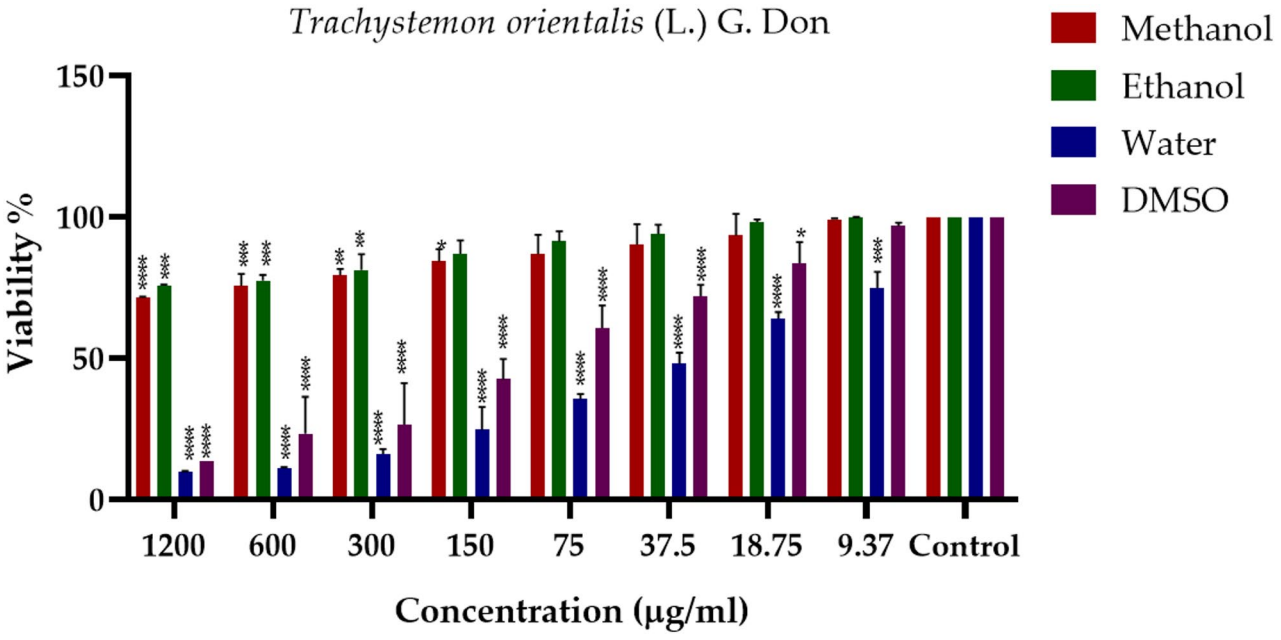

Fig. 1 MCF-7 cells were treated with TO extracts prepared in methanol, ethanol, DMSO, and distilled water at seven different concentrations (1200, 600, 300, 150, 75, 37.5, and 18.75 µg/mL). After 72 h of incubation, cell viability was evaluated using the MTT assay. Experiments were repeated twice, each with four technical replicates. Graphs were generated and statistical analyses were performed using GraphPad Prism 8.0.2, and differences between groups were evaluated using two-way ANOVA. In the graphs, *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$, ****$p < 0.0001$

Table 1 IC_{50} values of different extracts of TO and their selectivity indices

Solvent	IC_{50} Value (µg/mL)		SI
	MCF-7	CCD-1072sk	
Methanol	167.7 ± 4.15[c]	387.49 ± 2.19[c]	2.28 ± 0.1
Ethanol	185.30 ± 6.60[d]	446.89 ± 3.88[d]	2.46 ± 0.24
Water	28.88 ± 1.91[a]	63.38 ± 3.06 [a]	2.12 ± 1.19
DMSO	63.15 ± 5.42[b]	176.90 ± 0.42[b]	2.70 ± 1.15

($P < 0.001$). The IC_{50} values of the extracts in MCF-7 cells were water with 28.88 ± 1.91 µg/mL, DMSO with 63.15 ± 5.42 µg/mL, methanol with 167.70 ± 4.15 µg/mL, and ethanol extract with 185.30 ± 6.60 µg/mL, respectively. Corresponding IC_{50} values obtained from CCD-1072Sk normal cells were 63.38 ± 3.06 µg/mL for water, 176.90 ± 0.42 µg/mL for DMSO, 387.49 ± 2.19 µg/mL for methanol, and 446.89 ± 3.88 µg/mL for ethanol extracts. The selectivity indices (SI) were calculated as 2.12 ± 1.19, 2.70 ± 1.15, 2.28 ± 0.10, and 2.46 ± 0.24 for water, DMSO, methanol, and ethanol extracts, respectively (Table 1).

IC_{50} values were determined by cytotoxicity assays, and selectivity indices (SI) were calculated to evaluate the preferential toxicity of the extracts toward target cells. All experiments were performed in duplicate and repeated twice. Reported values represent the means ± standard de-viations of these replicates. Statistical analysis was performed using one-way ANOVA followed by Duncan's post hoc test. * There is no significant difference between groups with the same letters (a-d) in the same column.

RT-qPCR Results

Reactome databases were utilized to screen 92 target genes identified as being effective in breast cancer. Following the treatment with the aqueous extract of TO on MCF-7 breast cancer cells, the underlying potential biological mechanisms against breast cancer were explored. Among the target genes influenced by TO, a total of 77 genes were enriched within various signaling pathways (Fig. 2).

To determine the potential mechanisms of action, KEGG pathway enrichment analysis was performed using the ShinyGO 0.82 database. The KEGG analysis tree and bar plot revealed that TO primarily affected apoptosis-, cell cycle-, and cancer-related pathways. In addition, several key signaling pathways, including the PI3K–Akt, MAPK, p53, and HIF-1 signaling pathways, were significantly enriched (Fig. 3). These enriched pathways suggest that TO may influence multiple molecular processes involved in cancer cell survival, proliferation, and stress responses.

Gene expression studies conducted through GO (Gene Ontology) enrichment analysis on MCF-7 cancer cells treated with TO extract are also presented in Supplement Figs. 2, 3 and 4. GO biological process analysis revealed enrichment in DNA biosynthesis and programmed cell death, particularly in the regulation of apoptotic cell death (Supplementary Fig. 2). In the cellular component category, structures associated with the telomere and complexes related to the cell cycle were identified

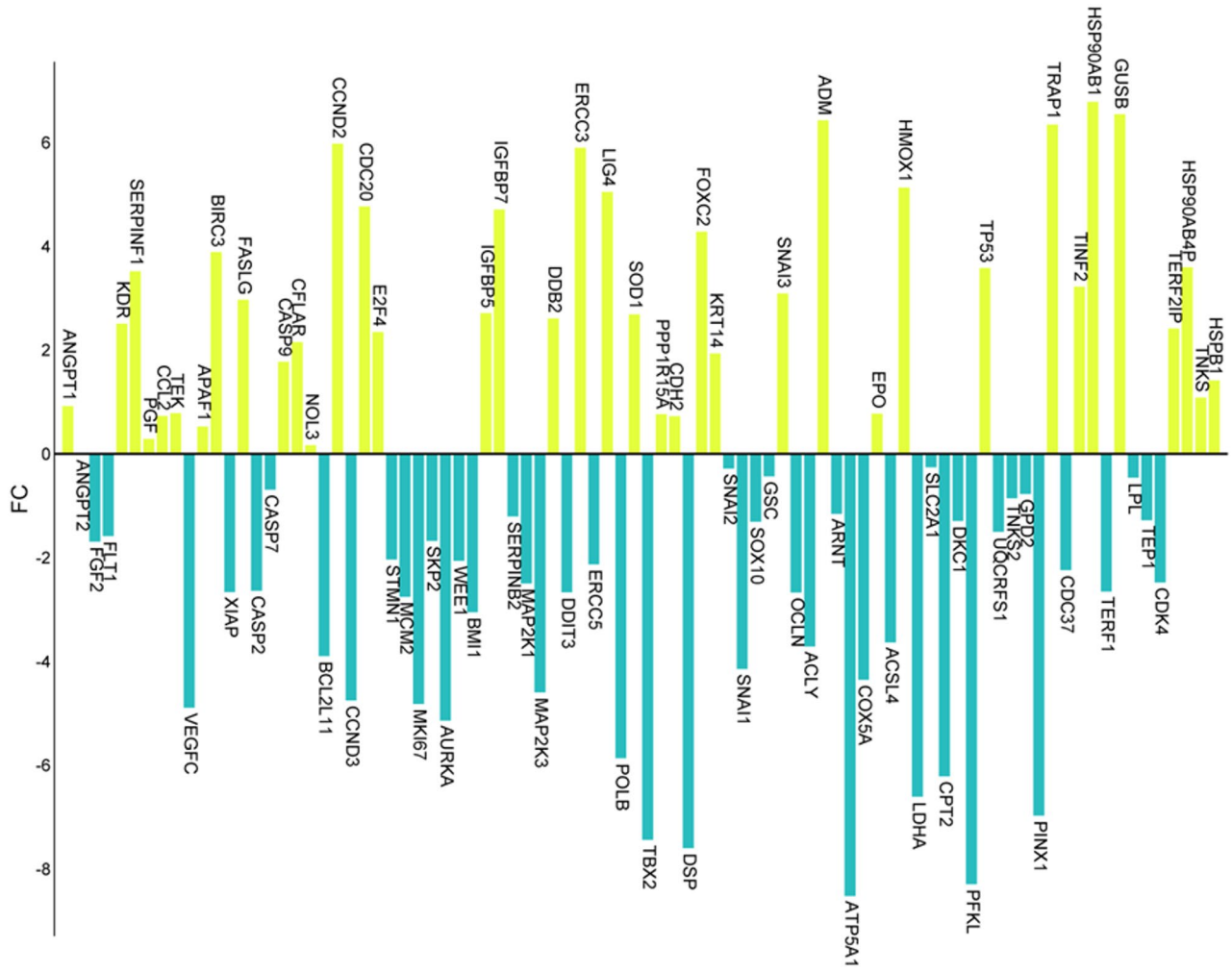

Fig. 2 Gene expression changes in signaling pathways influenced by TO

(Supplementary Fig. 3). In the molecular function category, terms related to telomere-associated processes, apoptosis, and various kinase activities were found to be significantly enriched (Supplementary Fig. 4).

Gene expression levels indicate the involvement of key metabolites in regulation. Gene enrichment analysis through EnrichR determines these metabolites (Table 2) and MetaboAnalyst 6.0 Network Explorer module. The relationship between genes and metabolites was determined through Network Explorer, and the result is shown in Fig. 4. Key metabolites including ADP, ATP, glycerol, palmitic acid, acetyl CoA, Coenzyme A and FAD regulate hub genes including *ACYL*, *ACSL4*, *COX5A*, *CPT2*, *DKC1*, *GPD2*, *GUSB*, *LDHA*, *LPL*, *PFKL*, *SLC2A1*,and *UQRFS1*. Thus, the extract of TO regulates metabolites and genes in a way that drives the cancer cells to apoptosis. Thus, the extracts provide valuable treatment methods for breast cancer.

The glycolysis metabolism is tightly controlled by the metabolites. Pyruvic acid controls *LDHA*, fructose 6-phosphate and fructose 1,6 phosphate controls PFKL, Dihydroxy-acetone phosphate controls *GPD2* and *PFKL*. Glycolysis intermediates also control the metabolites. *GPD2* regulates glycerol, ADP/ATP, FAD, and dihydroxyacetone phosphate (Fig. 4). This reciprocal regulation is provided by the extracts to overcome cancer cells metabolic disregulation. Thus, regulation of metabolite-gene expression provides a unique mechanism for cancer treatment.

Cell cycle analysis

The effects of TO extract on the cell cycle status of MCF-7 breast cancer cells were evaluated (Fig. 5; Table 3). MCF-7 breast cancer cells were treated with the extract at a concentration of 28.88 ng/µL for 72 h. Cell cycle distribution results indicated that treatment with the plant extract led to a significant increase in the percentage of cells in the S phase ($P < 0.05$), while there was a significant decrease in the G0&G1 and G2&M phases ($P < 0.01$). The percentage of MCF-7 cells in the S phase

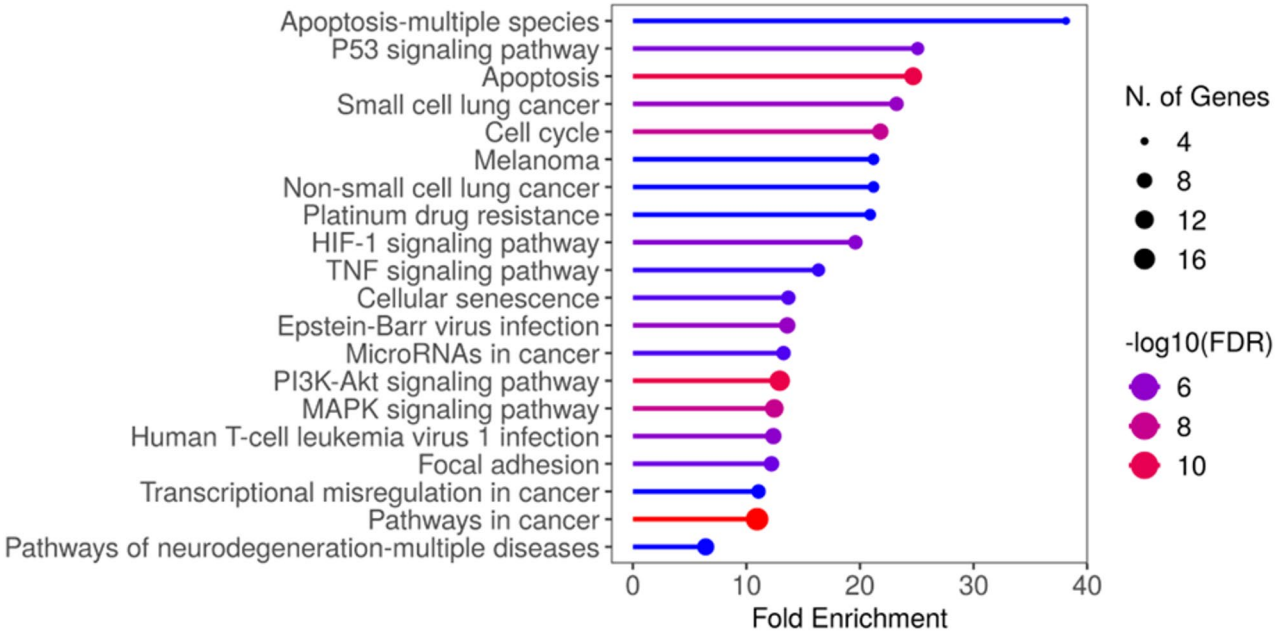

Fig. 3 KEGG pathway enrichment analysis bar plot of MCF-7 cancer cells treated with TO extract. KEGG analysis was performed on the ShinyGO website (v0.82) using genes that showed significant differential expression (accessed on June 26, 2025)

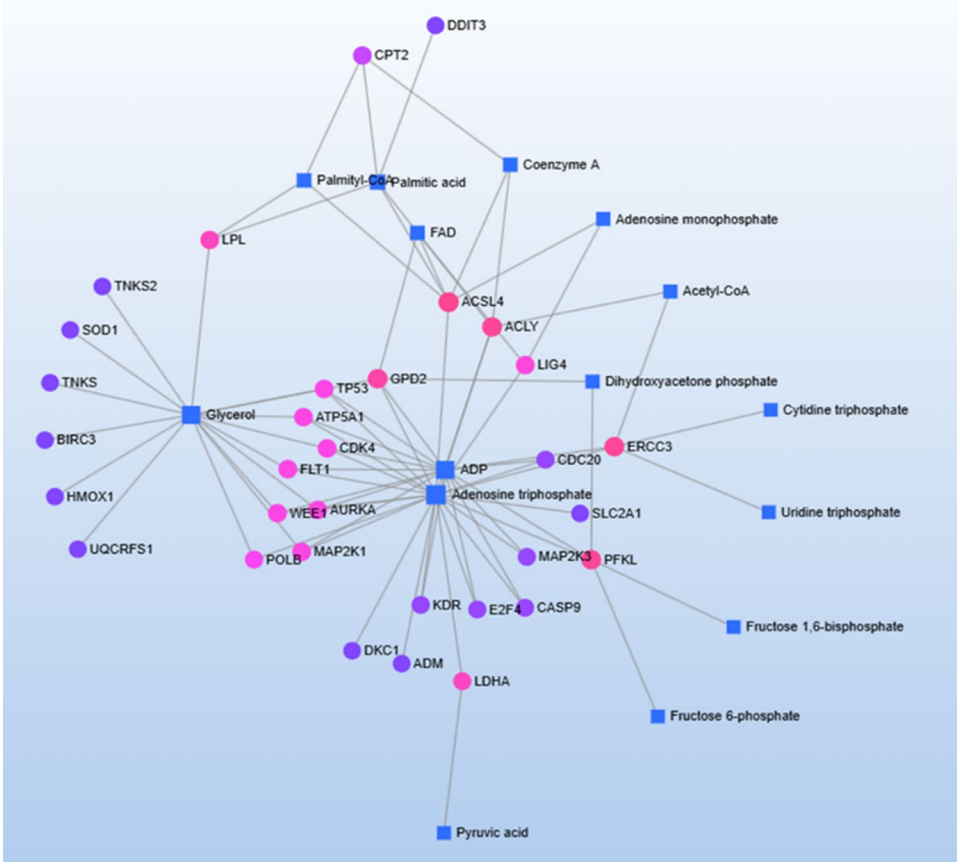

Fig. 4 Gene-metabolite analysis was performed using the MetaboAnalyst database to investigate genes with significant differential expression and their associated metabolites. The extracts influenced cancer mechanisms through key metabolites including glycerol, pyruvate, palmitic acid, ADP/ATP, FAD, acetyl-CoA, and coenzyme A. The data suggest that the extracts modulate the balance between metabolites and gene expression. Alterations in key metabolite levels were correlated with changes in hub gene expression, contributing to cell cycle arrest and apoptosis (accessed on March 10, 2025)

Table 2 Genes exhibiting significant differential expression were analyzed using the EnrichR program with the Metabolomics Workbench Metabolites database to identify related metabolites (accessed on March 10, 2025)

Index	Name	*P*-value	Adjusted *p*-value	Odds Ratio	Combined score
1	Palmitoyl-CoA	0.002398	0.05994	31.23	188.39
2	Coenzyme A	0.006603	0.06093	8.37	42.03
3	ATP	0.007312	0.06093	5.54	27.26
4	3-Mercaptopyruvic Acid	0.02288	0.06661	52.42	198.00
5	AMP	0.02529	0.06661	8.54	31.41
6	Triacylglycerol	0.02664	0.06661	43.68	158.34
7	Dihydroxyacetone Phosphate	0.02664	0.06661	43.68	158.34
8	Palmitic Acid	0.02664	0.06661	43.68	158.34
9	Fructose 1,6-Bisphosphate	0.02664	0.06661	43.68	158.34
10	Glycerol	0.02664	0.06661	43.68	158.34
11	sn-Glycero-3-phosphate	0.03039	0.06908	37.44	130.78
12	3-Methyl Pyruvic Acid	0.03413	0.07110	32.75	110.63
13	FAD	0.04892	0.08217	21.83	65.88
14	CDP	0.05259	0.08217	20.15	59.35
15	UTP	0.05259	0.08217	20.15	59.35
16	ITP	0.05259	0.08217	20.15	59.35
17	Fructose 6-Phosphate	0.05624	0.08270	18.71	53.85
18	IDP	0.05987	0.08316	17.46	49.17
19	CTP	0.06711	0.08830	15.41	41.62
20	Glucuronic Acid	0.07428	0.09268	13.78	35.84
21	Pyruvic Acid	0.07785	0.09268	13.09	33.43
22	ADP	0.09427	0.1071	4.00	9.44
23	Acetyl-CoA	0.1264	0.1374	7.70	15.92
24	UDP	0.2038	0.2123	4.51	7.17
25	NAD+	0.3389	0.3389	2.46	2.66

increased from 10.11 ± 1.12% (control) to 15.64 ± 0.60% following treatment with the extract. Conversely, the percentage of MCF-7 cells in the G2&M phase decreased from 23.01 ± 0.72% (control) to 20.74% ± 0.95%. Cells in the G0/G1 phase decreased significantly after plant extract treatment, with values of 66.74 ± 3.34% and 63.63 ± 3.18% ($P < 0.05$).

Apoptosis analysis

Flow cytometry analysis was performed to confirm the microscopic findings. As expected, treatment of MCF-7 breast cancer cells with the plant extract for 72 h resulted in significantly higher levels of apoptotic cells compared to the control group (control: 1.15%, treatment: 11.37%). Figure 6; Table 4 summarize the percentages of viable, apoptotic, and necrotic cells after treatment with TO extract. Table 4 presents the results of fluorescence intensity analysis of MCF-7 cells gated in Annexin V-FITC and PI channels. In each figure, the lower left quadrant represents viable (normal) cells, the lower right quadrant represents apoptotic (early apoptotic) cells, the upper right quadrant represents apoptotic (late apoptotic) cells, and the upper left quadrant represents necrotic cells. TO extract was found to induce apoptosis in MCF-7 cells. The percentages of viable, apoptotic and necrotic cells are summarized in Table 4. This evidence proved that TO extract was able to induce apoptosis in MCF-7 cells.

Cell imaging analysis

Cell nucleus, mitochondria, and skeletal structures were examined by staining 72 h after treatment of MCF-7 cells with TO aqueous extract at its IC50 value (Fig. 7). Compared to control cells, extract-treated cells exhibited subtle morphological alterations in nuclear and mitochondrial staining patterns. Nuclear staining suggested a tendency toward increased fluorescence intensity and a more rounded nuclear appearance. In addition, mitochondrial staining indicated changes in mitochondrial morphology, with structures appearing more elongated in some cells.

Evaluation of the merged images revealed morphological features suggestive of apoptotic processes. These observations indicate that treatment with the TO aqueous extract is associated with qualitative morphological alterations consistent with reduced cell viability.

Discussion

Many traditional plants have been proven to be an effective and safe source of pharmaceutical compounds in cancer treatment [2, 8, 10, 16, 17]. However, studies on the anti-cancer properties of TO are very limited. In a study, the TO plant showed a cytotoxic effect in the C6 glioblastoma cell line and similarly, when ethanol and water extracts were compared, it was shown that the water extract had a higher anti-proliferative effect [28]. In our study, the anti-tumor effect of TO aqueous extract was shown to be effective against MCF-7 breast cancer cells. Viability analyses revealed that the plant extract could inhibit cell proliferation in MCF-7 cells in a dose and treatment time-dependent manner. Furthermore, the cell proliferation inhibition effect of TO was found to be greater than that of *Moringa oleifera* seed [39], *Phyla nodiflora* L [40]., *Acalypha wilkesiana* [41] and *Artocarpus heterophyllus* [42], plants traditionally recognized for their effectiveness in cancer treatment. In a previous study conducted by researchers, the bioactive compound content of the TO plant was determined. It was found to contain phenolic compounds with anticancer effects, including caffeic acid, protocatechuic acid, and p-coumaric acid [18]. It is believed that the inhibitory effect of TO on cell proliferation is due to the bioactive compounds it contains.

This study also investigated the molecular mechanism of TO for the treatment of MCF-7 breast cancer cells.

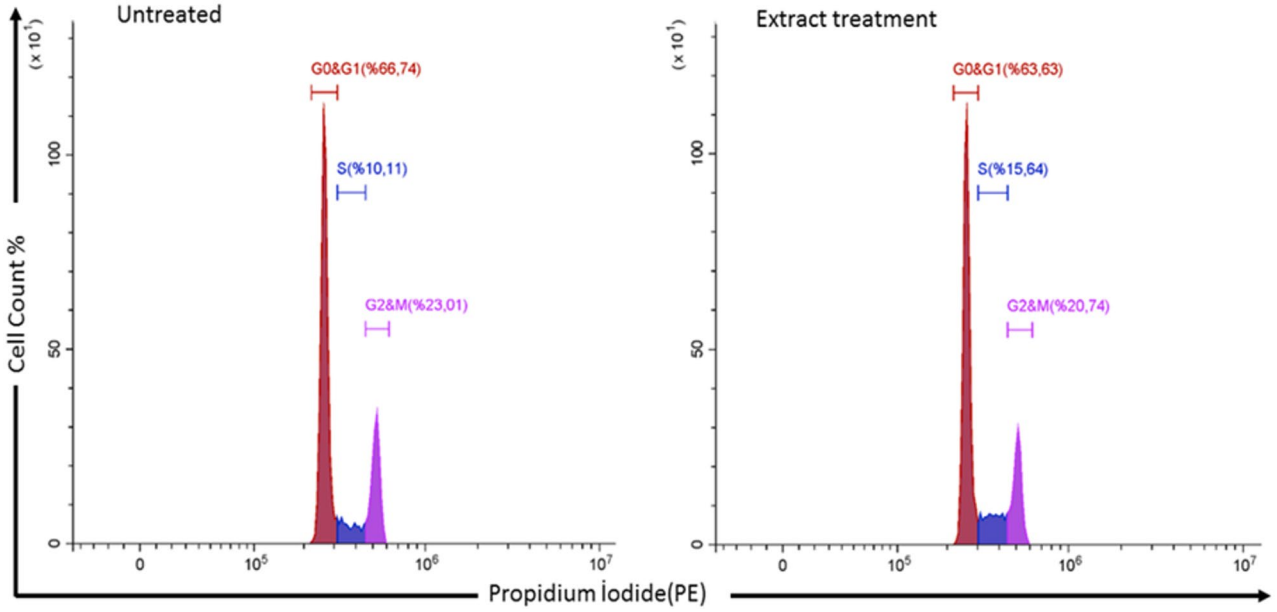

Fig. 5 The cell cycle analysis of MCF-7 cancer cells under control and TO-water treatments. Following staining with propidium iodide (PI), flow cytometry analysis was conducted to evaluate the distribution of cells across cell cycle phases. '*': P < 0,05, '**': P < 0,01,'***': P < 0,001

Table 3 Effect of TO extract on cell cycle checkpoints of MCF-7 breast cancer cells

Checkpoints in Cell Cycle	Control (%)	TO extract (%)
G0 & G1	66.74 ± 3.34	63.63 ± 3.18
S	10.11 ± 0.51	15.64 ± 0.78***
G2 & M	23.01 ± 1.15	20.74 ± 1.04

'*': P < 0,05, '**': P < 0,01,'***': P < 0,001

Table 4 Effect of TO extract on apoptosis of MCF-7 breast cancer cells

Signal Generation Areas	Control (%)	TO extract (%)
Live	97.38 ± 3.35	87.33 ± 4.37*
Early Apoptosis	0.78 ± 0.04	9.03 ± 0.45***
Late Apoptosis	0.37 ± 0.02	2.34 ± 0.12***
Necrosis	0.32 ± 0.02	1.30 ± 0.07***

'*': P < 0,05, '**': P < 0,01,'***': P < 0,001 indicates statistical significance compared to the control

KEGG and GO analyses were conducted to elucidate this mechanism. From the KEGG analysis, it was determined that TO affects multiple signaling pathways, including the HIF-1 signaling pathway, TNF signaling pathway,

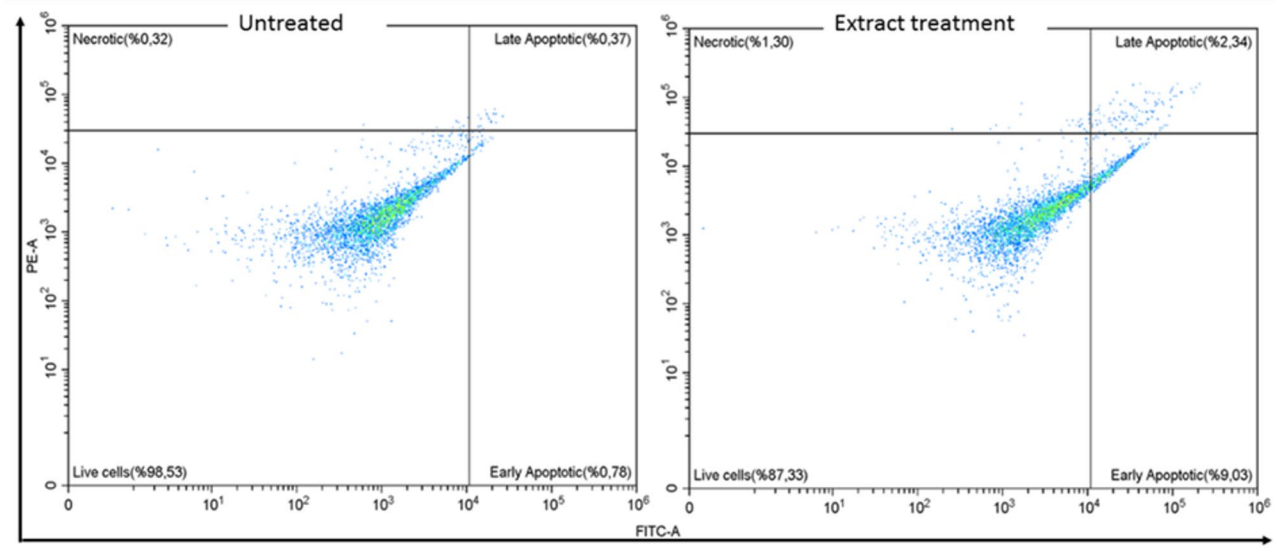

Fig. 6 Apoptosis in MCF-7 cancer cells by the treatment of control and TO-water extract. Annexin V-FITC/propidium iodide staining was used to calculate the percentages of apoptotic and necrotic cells.'*': P < 0,05, '**': P < 0,01,'***': P < 0,001 indicates statistical significance compared to the control

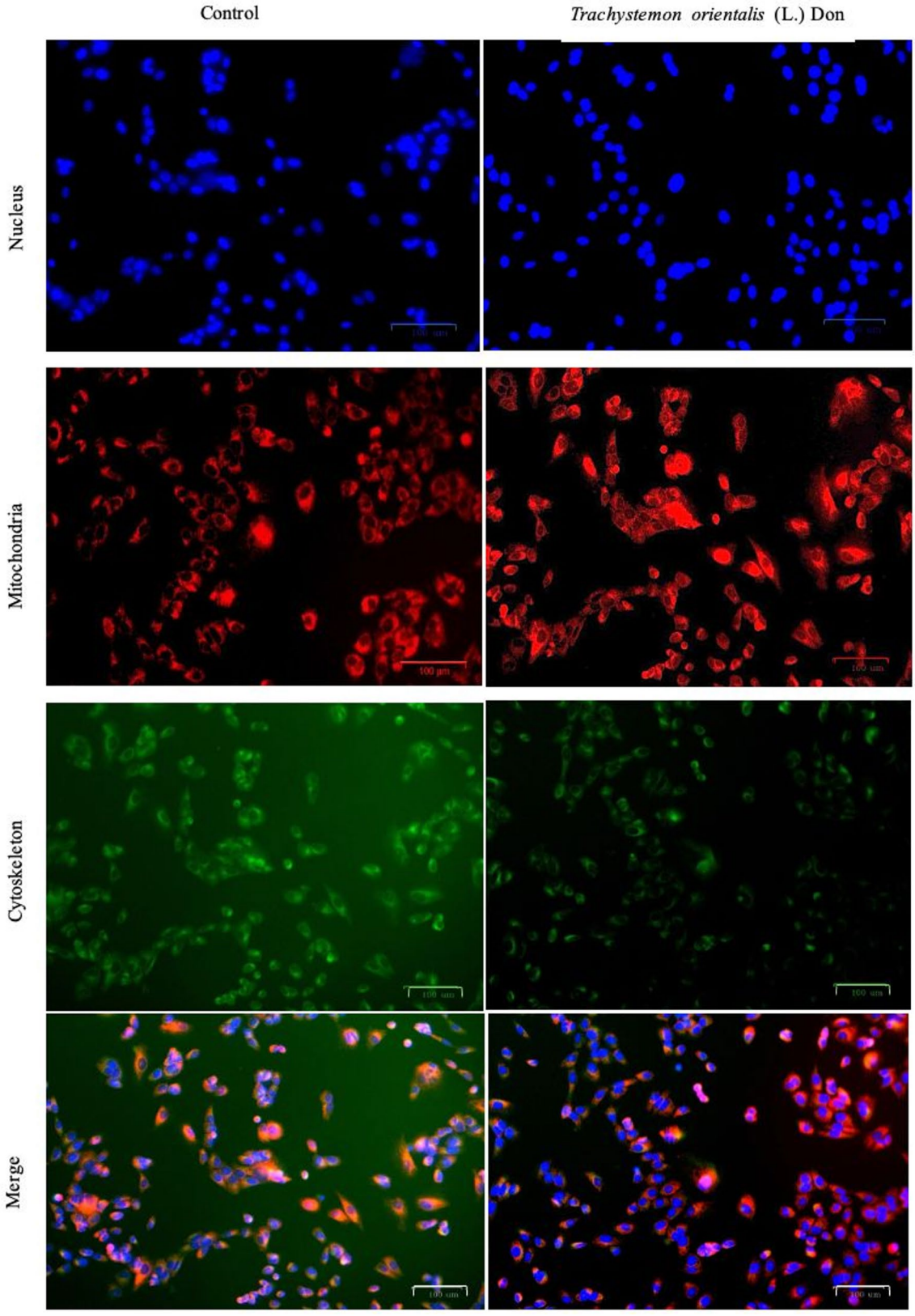

Fig. 7 Representative fluorescence pictures demonstrating morphological changes in MCF-7 breast cancer cells 72 h after the TO aqueous extract was administered at its IC_{50} concentration. To qualitatively evaluate changes in cell morphology, particular fluorescent dyes were used to visualize nuclear, cytoskeletal, and mitochondrial structures. Cells treated with extract showed characteristics that suggested apoptotic processes. Scale bar:100 µm

PI3K-Akt signaling pathway, MAPK signaling pathway, and p53 signaling pathway, all contributing to the inhibition of cancer cells.

Phosphatidylinositol 3-kinases (PI3Ks) are key coordinators of intracellular signaling in response to extracellular stimuli. Increased PI3K signaling is one of the most critical events in human cancers [43]. This abnormal upregulation can trigger cellular transformation, cancer cell development, progression, and the development of drug resistance [44]. The PI3K/AKT/mTOR pathway also plays a pivotal role in supporting the Warburg Effect observed in cancer cells. This pathway regulates both the expression of the *GLUT* family, which is responsible for glucose uptake, and enzymes involved in glycolysis. These actions enhance glycolytic activity, allowing cancer cells to bypass rate-limiting enzymes [45]. Additionally, *AKT* contributes to glycogen metabolism, an important energy storage source, thereby supporting the survival of cancer cells under nutrient-deprived conditions [46].

Recent studies have increasingly targeted the regulation of the PI3K-Akt signaling pathway due to its critical role in breast cancer. Specifically, in breast cancer, the pharmaceutical industry has focused extensively on this pathway, and it has been shown to block the apoptotic pathway [47–49]. Blocking the PI3K-Akt signaling pathway holds promise for the long-term treatment of breast cancer. Preventing the abnormal activation of the PI3K-Akt signaling pathway can help overcome resistance to breast cancer treatment [50]. Studies have demonstrated that certain plant extracts exhibit a significant antitumor effect by suppressing the PI3K-Akt signaling pathway [51, 52]. In this study, TO was found to exert its anticancer effects on breast cancer by regulating the PI3K-Akt signaling pathway.

Mitogen-activated protein kinase (MAPK), also known as *ERK*, is an enzyme that phosphorylates mitogen-activated proteins [50]. The MAPK signaling pathway plays a critical role in cell proliferation, growth, and apoptosis, and is heavily implicated in the formation and development of breast cancer [53]. Activation of the MAPK signaling pathway by oxidative stress can lead to apoptotic cell death and cell cycle arrest. The level of oxidative stress is a delicate balance for cancer cells; a balanced stress mechanism regulates cancer cell growth. When this balance is disrupted, it prevents the growth and proliferation of cancer cells by halting the cell cycle [54].

Lv et al. demonstrated that *Resina draconis* extract inhibited cell proliferation and stimulated the apoptotic pathway by weakening the MAPK signaling pathway in the MCF-7 breast cancer cell line [51]. Similarly, in our study, the extract from TO was found to stimulate apoptosis by affecting the MAPK signaling pathway. Especially as a result of GO enrichment analysis, it was found that apoptotic pathways were stimulated as a result of significant enrichment in biological process (BP) and molecular function (MC) and affected the cell cycle of cancer cells.

The aqueous extract of TO was found to be effective on the tumor suppressor gene p53 signaling pathway. The tumor suppressor gene p53 plays a pivotal role in mediating cellular responses by regulating the transcriptional activation of various genes involved in apoptosis, cell cycle arrest, and DNA repair [48]. Gene enrichment analyses revealed that TO was linked to molecular responses related to apoptosis and cytotoxicity in MCF-7 breast cancer cells. These analyses shed light on the potential mechanism of action of TO by identifying important signaling pathways and alterations in gene expression that may be involved in its cellular effects.

Hypoxia-Inducible Factor-1 (*HIF-1*) is a transcription factor involved in cellular processes such as angiogenesis, glucose metabolism, apoptosis, cell proliferation, and particularly oxygen homeostasis [55]. The hypoxic environment commonly observed in cancer cells leads to an increase in mitochondrial activities, resulting in elevated production of Reactive Oxygen Species (ROS). The increased levels of ROS stabilize HIF-1α by inhibiting its degradation, thereby activating the *HIF-1* pathway. This pathway, in turn, regulates the amount of ROS in the cell [56]. In our study, we demonstrated that the HIF-1 pathway is significantly affected by TO. The change in the *HIF-1* pathway may have caused the accumulation of ROS in the cell, leading to cell death.

Tumor Necrosis Factor (TNF) is a family of cytokines involved in cell survival, apoptosis, necrosis, inflammation, and the development of immune responses as part of the immune system [57]. The effect of the TNF family on cancer cells can be dual. Studies have shown that TNF may promote angiogenesis through chronic inflammation, thereby facilitating cancer progression. Conversely, it may also exhibit a cancer-suppressive role by inducing cellular death programs or through activation of the immune system [58]. In this study, gene enrichment analyses indicated that TO extract significantly affected the TNF pathway. This was supported by flow cytometry results, which showed an increase in necrosis. These findings suggest that TO may induce cell death through necrosis in breast cancer cells.

The cell cycle is an important regulator of cell division, growth and proliferation after DNA damage. It controls the transition from cell resting state (G0) to proliferation. It ensures correct genetic transcription through cell cycle checkpoints. The cell cycle is the mechanism of reproduction and is divided into four stages. These stages are G1 and G2, S phase (DNA synthesis) and M phase (mitosis) cycles [59]. This study shows that the plant extract prevents the proliferation of cancerous cells by arresting the cell cycle in MCF-7 breast cancer cells, providing a

strong example of tumor suppression. At the cell cycle checkpoint, the S checkpoint prevents the progression of the cell cycle by repairing damaged DNA within the cell. In various cancer cells, S phase cell cycle arrest has been shown to be induced in suppressing cancer cell proliferation. For example, a naturally occurring naphthoquinone isolated from *Phyla nodiflora* L. has been shown to play an important role in S phase cell cycle arrest [40]. In another study, the effects of ethanolic extract of *Mangifera indica* L. seed on lung cancer cells were investigated and it was reported that the extract induced cell cycle arrest at the S and G0/G1 checkpoint and caused apoptosis in cancer cells [60].

Moreover, a compound obtained from olive oil was reported to arrest the cell cycle at the S phase in breast cancer cells, leading to apoptosis [61]. In this study, it was deter-mined that the plant extract significantly inhibited the S phase, thereby suppressing MCF-7 cell growth.

Apoptosis is an important process in protecting uncontrolled proliferating cells against abnormal growth. One of the best methods to quantitatively analyze apoptosis is FITC-PI staining [62]. *Piper chaba* extract on breast cancer cells, arrested the cell cycle at the G0/G1 phase and induce apoptosis [63]. In a study on MCF-7 breast cancer cells with *Momordica balsamina* extract, similar results were observed and it was determined that apoptosis of cancerous breast cells was induced by the plant extract [64]. In line with these findings, our study demonstrated that the TO extract induced apoptosis in MCF-7 cells. These results are consistent with earlier studies, further confirming that plant extracts have the ability to induce apoptosis [63, 64]. Previous studies have reported that nuclear structures appear brighter in Hoechst 33,342 staining due to DNA damage [65, 66]. Similarly, in this study, the observed increase in nuclear fluorescence suggests that extract application may lead to nuclear structural disruption. Changes in mitochondrial morphology have generally been associated with metabolic disturbances [67, 68]. In our study, alterations in mitochondrial morphology were also observed, suggesting a potential impact on cellular metabolism. Furthermore, consistent with our apoptosis results, apoptotic cell morphology was observed following extract application.

The extract helps regulate metabolite-gene expression in cancer cells. The control of glycolysis energy metabolism through the extracts weakens cancer cells especially glycolysis and glycolysis intermediates through metabolites. This study suggests that TO extract may modulate gene expression profiles, as indicated by exploratory array analysis, and these molecular changes are supported by functional cell cycle, apoptosis, and cell imaging assays in breast cancer cell lines. The extract provides a unique mechanism for cancer treatment and elucidates the molecular mechanism at the gene-metabolite level.

Limitation of the Study

The current study employs a single cancer cell line (MCF-7) along with one healthy fibroblast cell line. Although these cells are well established, they may not reflect the complexity of breast cancer biology in vivo. Thus, the findings may not be extrapolated to physiological or clinical conditions without animal models and clinical studies.

The mechanistic conclusions mostly depend on transcriptomic changes and pathway enrichment analysis. Although KEGG, GO, and gene–metabolite network analyses provide valuable insights into systems as a whole, they do not immediately indicate whether a pathway is activated or deactivated. Crucial signaling pathways were not validated at the protein or phosphorylation level, hence limiting definitive mechanistic interpretations. It should be noted that there are several variables in the biochemical pathways and the enrichment analysis provides an integrative approach through examining gene-metabolite enhancement.

The study employed a crude aqueous extract of *Trachystemon orientalis* and this may limit the study as standardized bioactive constituents were not used. However, the *Trachystemon orientalis* plant was used as a whole to cook as a traditional treatment. This is why the study employs the extract but isolation or standardized bioactive constituents may be more beneficial.

The present study also did not examine pharmacokinetics, bioavailability or systemic toxicity for assessing treatment feasibility. Therefore, the presented results are exploratory and hypothesis-generating, thus additional molecular validation, compound(s) isolation and in vivo experiments must be performed to fully understand the molecular effect.

Conclusions

In conclusion, the present study demonstrates that the TO aqueous extract exerts anticancer-related effects on MCF-7 breast cancer cells, as evidenced by reduced cell viability, flow cytometry–based alterations in apoptosis and cell cycle distribution, and qualitative morphological changes. These experimental findings indicate that TO treatment negatively affects cancer cell survival and proliferation under in vitro conditions.

Complementary silico analyses, including transcriptome profiling and enrichment of KEGG pathways, suggested that multiple signalling pathways associated with tumour-related disease, such as apoptosis, regulation of the cell cycle, PI3K-Akt, MAPK, p53 and HIF-1, are involved. The integration of experimental tests with computational pathogenicity analysis provides a multilayered framework to help explain the observed cellular effects of TO water extract.

However, as several mechanistic interpretations are derived primarily from omics and pathway enrichment analyses, the results should be considered as exploratory and hypothesis-based rather than conclusive mechanistic. Future studies should focus on validating these findings through targeted gene and protein expression analyses, functional pathway modulation, and expanded biological replicates. Overall, the findings of this study support the potential of the TO aqueous extract as a candidate subject to further investigation and validation in breast cancer research, rather than as a direct therapeutic agent.

Abbreviations

TO	Trachystemon orientalis (L.) G. Don
DMSO	Dimethyl sulfoxide
NCD	Non-communicable diseases
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal bovine serum
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide
SI	Selectivity index
PI3Ks	Phosphatidylinositol 3-kinases
MAPK	Mitogen-activated protein kinase
TNF	Tumor Necrosis Factor
HIF-1	Hypoxia-Inducible Factor-1

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12906-026-05334-1.

Supplementary Material 1

Acknowledgements

Ayse Büsranur Celik acknowledges the Recep Tayyip Erdoğan University Development Foundation for awarding her the doctoral scholarship.

Authors' contributions

Conceptualization, Y.D.O. and Y.E.Y.; methodology, Y.D.O, L.G., A.B.C, O.S., L.T. and Y.T.; software, Y.D.O.; formal analysis, Y.D.O; investigation, Y.D.O. and Y.E.Y.; resources, Y.D.O, L.G., A.B.C, O.S., L.T. and Y.T.; data curation, Y.D.O.; writing—original draft preparation, Y.D.O, L.G., A.B.C, O.S., L.T. and Y.T.; writing—review and editing, Y.D.O, L.G., A.B.C, O.S., L.T. and Y.T.; visualization, Y.T; supervision, Y.T; project administration, Y.D.O.; All authors read and approved the final manuscript.

Funding

This study has been supported by the Recep Tayyip Erdogan University Development Foundation (Grant Number: 02025011017808) and TUBITAK 2209-A Program (Project No: 1919B012322527).

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study does not involve any human or animal testing. The figures created by Reactome were used in line with their policies. Sampling of Trachystemon orientalis was conducted non-destructively from a publicly accessible area (Kurtuluş village, Rize; 41.0351199, 40.5929884) in compliance with local regulations; no specific permit was required.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Nutrition and Dietetics, Faculty of Health Sciences, Recep Tayyip Erdoğan University, Rize, Turkey
[2]Department of Plant and Animal Production, Mudurnu Süreyya Astarcı Vocational College, Bolu Abant İzzet Baysal University, Bolu, Türkiye, Turkey
[3]Department of Molecular Biology and Genetics, Health Sciences Institutes, Health Sciences University, Istanbul, Türkiye, Turkey
[4]Division of Medicinal Biochemistry, Department of Basic Medical Sciences, Faculty of Medicine, Recep Tayyip Erdogan University, Rize 53020, Türkiye, Turkey
[5]Department of Nursing, Faculty of Health Sciences, Recep Tayyip Erdoğan University, Rize, Türkiye, Turkey
[6]Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Kırşehir Ahi Evran University, Kırsehir, Türkiye
[7]Training and Research Hospital, Recep Tayyip Erdoğan University, Rize 53020, Turkey

Received: 18 August 2025 / Accepted: 4 March 2026
Published online: 07 March 2026

References

1. Zarredar H, Khamaneh AM, Amoodizaj FF, Shanehbandi D, Seyedrezazadeh E, Jadid HS, et al. *Terminalia catappa* extract (TCE) reduces proliferation of lung and breast cancer cell by modulating miR-21 and miR-34a expressions. Asian Pac J Cancer Prev. 2021. https://doi.org/10.1002/cam4.70986.
2. Mathiyazhagan J, Kodiveri Muthukaliannan G. Combined *Zingiber officinale* and *Terminalia chebula* induces apoptosis and modulates mTOR and hTERT gene expressions in MCF-7 Cell Line. Nutr Cancer. 2021. https://doi.org/10.1080/01635581.2020.1792518.
3. Abbas P, Samsudin N, Ishak NI, Salleh HM, Azziz SSSA, Al-Khatib MAFR, et al. Agarwood branch ethanolic extract effects expression of epoptotic genes in MCF-7 breast cancer cells. Prog Drug Discov Biomed Sci. 2021. https://doi.org/10.36877/pddbs.a0000239.
4. Dembic Z. Antitumor drugs and their targets. Molecules. 2020. https://doi.org/10.3390/molecules25235776.
5. Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer. 2021. https://doi.org/10.1002/cncr.33587.
6. Guida F, Kidman R, Ferlay J, Schüz J, Soerjomataram I, Kithaka B, et al. Global and regional estimates of orphans attributed to maternal cancer mortality in 2020. Nat Med. 2022. https://doi.org/10.1038/s41591-022-02109-2.
7. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024. https://doi.org/10.3322/caac.21834.
8. Farshori NN, Siddiqui MA, Al-Oqail MM, Al-Sheddi ES, Al-Massarani SM, Saquib Q, et al. *Aloe vera*-induced apoptotic cell death through ROS generation, cell cycle arrest, and DNA damage in human breast cancer cells. Biologia. 2022. https://doi.org/10.1007/s11756-022-01124-5.
9. Wen X, Guo X, Wang S, Lu Z, Zhang Y. Breast cancer diagnosis a systematic review. Biocybern Biomed Eng. 2024. https://doi.org/10.1016/j.bbe.2024.01.002.
10. Fawzy RM, Abdel-Aziz AA, Bassiouny K, Fayed AM. Phytocompounds-based therapeutic approach: investigating Curcumin and Green Tea Extracts on MCF-7 breast cancer cell line. J Genet Eng Biotechnol. 2024. https://doi.org/10.1016/j.jgeb.2023.100339.
11. Al-Harbi LN, Subash-Babu P, Binobead MA, Alhussain MH, Alsedairy SA, Aloud AA, et al. Potential metabolite nymphayol isolated from water lily (*Nymphaea stellata*) flower inhibits MCF-7 human breast cancer cell growth via upregulation of CDKN2A, PRB2, P53 and downregulation of PCNA mRNA expressions. Metabolites. 2020. https://doi.org/10.3390/metabo10070280.
12. Tohkayomatee R, Reabroi S, Tungmunnithum D, Parichatikanond W, Pinthong D. Andrographolide exhibits anticancer activity against breast cancer cells (MCF-7 and MDA-MB-231 Cells) through suppressing cell proliferation and inducing cell apoptosis via inactivation of ER-α receptor and PI3K/AKT/mTOR signaling. Molecules. 2022. https://doi.org/10.3390/molecules27113544.

13. Razali S, Firus Khan AY, Khatib A, Ahmed QU, Abdul Wahab R, Zakaria ZA. An in vitro anticancer activity evaluation of *Neolamarckia cadamba* (Roxb.) Bosser leaves' extract and its metabolite profile. Front Pharmacol. 2021. https://doi.org/10.3389/fphar.2021.741683.
14. Ahmad I, Irfan S, Dera AA, Zaman GS, Chandramoorthy HC, Mir MM, et al. GC-MS analysis of ethanol extract from areal parts of *Nepeta deflersiana* and its anticancer and antimicrobial efficacies. Biologia. 2020. https://doi.org/10.2478/s11756-020-00473-3.
15. Rizeq B, Gupta I, Ilesanmi J, AlSafran M, Rahman MM, Ouhtit A. The power of phytochemicals combination in cancer chemoprevention. J Cancer. 2020. https://doi.org/10.7150/jca.34374.
16. Rameshbabu S, Messaoudi SA, Alehaideb ZI, Ali MS, Venktraman A, Alajmi H, et al. *Anastatica hierochuntica* (L.) methanolic and aqueous extracts exert antiproliferative effects through the induction of apoptosis in MCF-7 breast cancer cells. Saudi Pharm J. 2020;28:985–93. https://doi.org/10.1016/j.jsps.2020.06.020.
17. Salam HS, Tawfik MM, Elnagar MR, Mohammed HA, Zarka MA, Awad NS. Potential apoptotic activities of *Hylocereus undatus* peel and pulp extracts in MCF-7 and Caco-2 cancer cell lines. Plants. 2022. https://doi.org/10.3390/plants11172192.
18. Demirel Ozbek Y, Saral O, Turker PF. Modern and traditional cooking methods affect the antioxidant activity and phenolic compounds content of *Trachystemon orientalis* (L.) G. Don. PLoS One. 2024. https://doi.org/10.1371/journal.pone.0299037.
19. Turfan N. Variation of minerals content and some bioactive compound in some wild and cultivated edible plants grown naturally in Kastamonu region. J Inst Sci Technol. 2021. https://doi.org/10.21597/jist.893796.
20. Biyik B, Sarialtin SY, Gökbulut A, Çoban T, Coşkun M. *Trachystemon orientalis* (L.) G. Don as a valuable source of rosmarinic acid: biological activities and HPLC profiles. Turk J Pharm Sci. 2023. https://doi.org/10.4274/tjps.galenos.2022.14265.
21. Sacan O. Antioxidant activity, total phenol and total flavonoid contents of *Trachystemon orientalis* (L.) G. Don. Eur J Biol. 2018. https://doi.org/10.26650/EurJBiol.2018.18017.
22. Dimcheva V, Kaloyanov N, Karsheva M. The polyphenol composition of *Cistus incanus* L., *Trachystemon orientalis* L. and *Melissa officinalis* L. infusions by HPLC-DAD method. Open J Anal Bioanal Chem. 2019. https://doi.org/10.17352/ojabc.000008.
23. Demir E. Profile of fatty acids, vitamins, phytosterols and phenolic acids in *Trachystemon orientalis* L. and evaluation of their antioxidant activity. EJOSAT. 2022. https://doi.org/10.31590/ejosat.1027061.
24. Onaran A, Yılar M. Antifungal activity of *Trachystemon orientalis* L. aqueous extracts against plant pathogens. J Food Agric Environ. 2012;10:287–91.
25. Ayhan BS, Yalcın E, Cavusoglu K, Acar A. Antidiabetic potential and multi-biological activities of *Trachystemon orientalis* extracts. J Food Meas Charact. 2019. https://doi.org/10.1007/s11694-019-00209-1.
26. Kurtulus G, Toksoz O, Sesal C. Potential application of *Trachystemon orientalis* L. extracts in the cosmetic industries: Skincare, photoprotective and antiaging ingredients. Int J Adv Eng Pure Sci. 2022. https://doi.org/10.7240/jeps.1056274.
27. Aksu MI, Derman H. The effects of water, methanol and petrolium ether extracts of *Trachystemon orientalis* L. on some quality properties and shelf life of ground beef. J Food. 2022. https://doi.org/10.15237/gida.GD22023.
28. Al Yassine D, El Massri N, Demircan G, Bulut G, Akin D, Tacer-Caba Z. Total antioxidant potential, total phenolic profile and cytotoxic activity against brain cancer: melocan and galdirik. Food Technol Biotechnol. 2023. https://doi.org/10.17113/ftb.61.04.23.8071.
29. Gülüm L, Güler E, Zırhlı ÇB, Çelik AB, Tutar Y. Phytochemical profiling and anticancer potential of pistachio wastes against MCF-7 breast cancer cells: a metabolic and apoptotic pathway analysis. BMC Complement Med Ther. 2025. https://doi.org/10.1186/s12906-025-04963-2.
30. Gülüm L, Güler E, Aktaş FL, Çelik AB, Yılmaz H, Tutar Y. In vitro effects of *Rumex confertus* extracts on cell viability and molecular pathways in MCF-7 breast cancer cells. Antioxidants. 2025. https://doi.org/10.3390/antiox14070879.
31. Gülüm L, Güler E, Çapkınoğlu E, Çelik AB, Tutar Y. Evaluation of anticancer potential of *Ganoderma lucidum* on MCF-7 breast cancer cells through genetic transcription of energy metabolism. Antioxidants. 2025. https://doi.org/10.3390/antiox14121471.
32. Lica JJ, Wieczór M, Grabe GJ, Heldt M, Jancz M, Misiak M, Gucwa K, Brankiewicz W, Maciejewska N, Stupak A. Effective drug concentration and selectivity depends on fraction of primitive cells. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms22094931.
33. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2 – ΔΔCT method. Methods. 2001. https://doi.org/10.1006/meth.2001.1262.
34. Ge SX, Jung D, Yao R. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinformatics. 2020. https://doi.org/10.1093/bioinformatics/btz931.
35. Xie Z, Bailey A, Kuleshov MV, Clarke DJB, Evangelista JE, Jenkins SL, et al. Gene set knowledge discovery with Enrichr. Curr Protoc. 2021. https://doi.org/10.1002/cpz1.90.
36. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016. https://doi.org/10.1093/nar/gkw377.
37. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: interactive and collab-orative HTML5 gene list enrichment analysis tool. BMC Bioinformatics. 2013. https://doi.org/10.1186/1471-2105-14-128.
38. Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, et al. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 2018. https://doi.org/10.1093/nar/gky310.
39. Adebayo IA, Arsad H, Samian MR. Antiproliferative effect on breast cancer (MCF7) of *Moringa oleifera* seed extracts. Afr J Tradit Complement Altern Med. 2017. https://doi.org/10.21010/ajtcam.v14i2.30.
40. Teoh PL, Liau M, Cheong BE. *Phyla nodiflora* L. extracts induce apoptosis and cell cycle arrest in human breast cancer cell line, MCF-7. Nutr Cancer. 2019. https://doi.org/10.1080/01635581.2018.1559942.
41. Halimah E, Hendriani R, Indradi B, Ferdiansyah F, Sofian FF. Cytotoxicity of ethanol extract and its fractions from *Acalypha wilkesiana* against breast cancer cell MCF-7. J Adv Pharm Educ Res. 2022. https://doi.org/10.51847/G2bMkvc6PO.
42. Patel RM, Patel SK. Cytotoxic activity of methanolic extract of *Artocarpus heterophyllus* against A549, Hela and MCF-7 cell lines. J Appl Pharm Sci. 2011;1:167–71.
43. Kalpage HA, Wan J, Morse PT, Zurek MP, Turner AA, Khobeir A, et al. Cytochrome c phosphorylation: control of mitochondrial electron transport chain flux and apoptosis. Int J Biochem Cell Biol. 2020. https://doi.org/10.1016/j.biocel.2020.105704.
44. Noorolyai S, Shajari N, Baghbani E, Sadreddini S, Baradaran B. The relation between PI3K/AKT signalling pathway and cancer. Gene. 2019. https://doi.org/10.1016/j.gene.2019.02.076.
45. Hoxhaj G, Manning BD. The PI3K–AKT network at the interface of oncogenic signalling and cancer metabolism. Nat Rev Cancer. 2019. https://doi.org/10.1038/s41568-019-0216-7.
46. Glaviano A, Foo AS, Lam HY, Yap KC, Jacot W, Jones RH, Eng H, Nair MG, Makvandi P, Geoerger B. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Mol Cancer. 2023. https://doi.org/10.1186/s12943-023-01827-6.
47. Guerrero-Zotano A, Mayer IA, Arteaga CL. PI3K/AKT/mTOR: role in breast cancer progression, drug resistance, and treatment. Cancer Metastasis Rev. 2016. https://doi.org/10.1007/s10555-016-9637-x.
48. Costa RLB, Han HS, Gradishar WJ. Targeting the PI3K/AKT/mTOR pathway in triple-negative breast cancer: a review. Breast Cancer Res Treat. 2018. https://doi.org/10.1007/s10549-018-4697-y.
49. Tewari D, Patni P, Bishayee A, Sah AN, Bishayee A. Natural products targeting the PI3K-Akt-mTOR signaling pathway in cancer: a novel therapeutic strategy. Semin Cancer Biol. 2022. https://doi.org/10.1016/j.semcancer.2019.12.008.
50. Kim GD. Ursolic acid decreases the proliferation of MCF-7 cell-derived breast cancer stem-like cells by modulating the ERK and PI3K/AKT signaling pathways. Prev Nutr Food Sci. 2021. https://doi.org/10.3746/pnf.2021.26.4.434.
51. Lv Y, Mou Y, Su J, Liu S, Ding X, Yuan Y, Li G. The inhibitory effect and mechanism of Resina Draconis on the proliferation of MCF-7 breast cancer cells: a network pharmacology-based analysis. Sci Rep. 2023. https://doi.org/10.1038/s41598-023-30585-0.
52. Ajji PK, Binder MJ, Walder K, Puri M. Balsamin induces apoptosis in breast cancer cells via DNA fragmentation and cell cycle arrest. Mol Cell Biochem. 2017. https://doi.org/10.1007/s11010-017-3009-x.
53. Khojasteh Poor F, Keivan M, Ramazii M, Ghaedrahmati F, Anbiyaiee A, Panahandeh S, et al. Mini review: the FDA-approved prescription drugs that target the MAPK signaling pathway in women with breast cancer. Breast Dis. 2021. https://doi.org/10.3233/BD-201063.
54. Ryu M, Sung CK, Im YJ, Chun C. Activation of JNK and p38 in MCF-7 cells and the in vitro anticancer activity of Alnus hirsuta extract. Molecules. 2020. https://doi.org/10.3390/molecules25051073.

55. Xia W, Gong E, Lin Y, Zheng B, Yang W, Li T, Zhang S. Wild pink bayberry free phenolic extract induces mitochon-dria-dependent apoptosis and G0/G1 cell cycle arrest through p38/MAPK and PI3K/Akt pathway in MDA-MB-231 cancer cells. Food Sci Hum Wellness. 2023. https://doi.org/10.1016/j.fshw.2023.02.014.
56. Galanis A, Pappa A, Giannakakis A, Lanitis E, Dangaj D, Sandaltzopoulos R. Reactive oxygen species and HIF-1 sig-nalling in cancer. Cancer Lett. 2008. https://doi.org/10.1016/j.canlet.2008.02.028.
57. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol. 2003. https://doi.org/10.1038/nri1184.
58. Wang X, Lin Y. Tumor necrosis factor and cancer, buddies or foes? Acta Pharmacol Sin. 2008. https://doi.org/10.1111/j.1745-7254.2008.00889.x.
59. Xia Y, Choi HK, Lee K. Recent advances in hypoxia-inducible factor (HIF)-1 inhibitors. Eur J Med Chem. 2012. https://doi.org/10.1016/j.ejmech.2012.01.033.
60. Alshwyeh HA, Al-Sheikh WMS, Rasedee A, Alnasser SM, Al-Qubaisi MS, Ibrahim WN. *Mangifera indica* L. kernel ethanol extract inhibits cell viability and proliferation with induction of cell cycle arrest and apoptosis in lung cancer cells. Mol Cell Oncol. 2024. https://doi.org/10.1080/23723556.2023.2299046.
61. Messeha SS, Zarmouh NO, Asiri A, Soliman KFA. Gene expression alterations associated with oleuropein-induced antiproliferative effects and S-phase cell cycle arrest in triple-negative breast cancer cells. Nutrients. 2020. https://doi.org/10.3390/nu12123755.
62. Rai N, Gupta P, Verma A, Tiwari RK, Madhukar P, Kamble SC, et al. Ethyl acetate extract of *Colletotrichum gloeosporioides* promotes cytotoxicity and apoptosis in human breast cancer cells. ACS Omega. 2023. https://doi.org/10.1021/acsomega.2c05746.
63. Prajapati KS, Kumar S. *Piper chaba*, an Indian spice plant extract, inhibits cell cycle G1/S phase transition and induces intrinsic apoptotic pathway in luminal breast cancer cells. Cell Biochem Funct. 2023. https://doi.org/10.1002/cbf.3857.
64. Serala K, Malemela KM, Boshielo IT, Riedel S, Mampuru L, Mbazima V, et al. *Momordica balsamina* acetone leaf extract induces apoptosis and inhibits the invasiveness and migration of MCF-7 breast cancer cells. S Afr J Bot. 2024. https://doi.org/10.1016/j.sajb.2023.12.043.
65. Li G, Wu X, Sun P, Zhang Z, Shao E, Mao J, Cao H, Huang H. Dithiolation indolizine exerts viability suppression effects on A549 cells via triggering intrinsic apoptotic pathways and inducing G2/M phase arrest. Biomed Pharmacother. 2020. https://doi.org/10.1016/j.biopha.2020.110961.
66. Sun Y, Wu P, Sun Y, Sharopov FS, Yang Q, Chen F, Wang P, Liang Z. Lycorine possesses notable anticancer potentials in on-small cell lung carcinoma cells via blocking Wnt/β-catenin signaling and epithelial-mesenchymal transition (EMT). Biochem Biophys Res Commun. 2018. https://doi.org/10.1016/j.bbrc.2017.11.032.
67. Baek ML, Lee J, Pendleton KE, Berner MJ, Goff EB, Tan L, Martinez SA, Mahmud I, Wang T, Meyer MD. Mitochondrial structure and function adaptation in residual triple negative breast cancer cells surviving chemotherapy treatment. Oncogene. 2023. https://doi.org/10.1038/s41388-023-02596-8.
68. Li S, Cheng H, Xie B, Qiu W, Li C, Li B, Cheng H, Zhang X. Mitochondria targeted cancer therapy using ethidium derivatives. Mater Today Chem. 2017. https://doi.org/10.1016/j.mtchem.2017.09.002.

Publisher's Note