İSTANBUL UNIVERSITY PRESS

Experimed 2026, 16 (1): 51–60

https://doi.org/10.26650/10.26650/experimed.1823647

Submitted: 17.11.2025
Revision Requested: 03.02.2026
Last Revision Received: 14.03.2026
Accepted: 10.04.2026
Published Online 28.04.2026

Experimed

Research Article

Open Access

Transcriptomic Profiling Reveals Cell Cycle and DNA Repair Pathway Modulation in Triple-Negative Breast Cancer Cells Exposed to Polyphenol-rich Propolis

Mehmet Fatih Seyhan [1], Özlem Timirci Kahraman [2], Naci Şenkal [3], Halil İbrahim Kısakesen [4], Allison Pınar Eronat [5], Tülin Öztürk [6], Fatih Yanar [7], Hülya Yılmaz Aydoğan [2] & Oğuz Öztürk [2]

[1] İstanbul Yeni Yüzyıl University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, İstanbul, Türkiye
[2] İstanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Molecular Medicine, İstanbul, Türkiye
[3] İstanbul, University, Faculty of Medicine, Department of Internal Medicine, İstanbul, Türkiye
[4] İstanbul, Technical University, Faculty of Science, Department of Molecular Biology and Genetics, İstanbul, Türkiye
[5] Halic University, Department of Molecular Biology and Genetics, İstanbul, Türkiye
[6] İstanbul University-Cerrahpasa, Faculty of Medicine, Department of Pathology, İstanbul, Türkiye
[7] Boğazici University, Department of Molecular Biology and Genetics, İstanbul, Türkiye

Abstract

Objective: Triple-negative breast cancer (TNBC) is the most invasive subtype of breast cancer distinguished by its high recurrence rates and clinical aggression. Honey bees naturally create propolis, which can be used as an alternative therapy for breast cancer due to its anti-cancer properties. The study aimed to examine the impacts of propolis by elucidating potential anticarcinogenic and modulatory effects focusing on whole-genome expression levels.

Materials and Methods: This study aimed to evaluate the effects of 50 µg/mL propolis concentration for 48 and 72-hours on gene expression levels in MDA-MB-231 cells using microarray assay. Microarray results were also validated using selected genes such as BIRC5, CDC25C, CCN1, CCN2, and CDK1 via the quantitative reverse transcription polymerase chain reaction (qRT-PCR) method.

Results: According to bioinformatics analysis, 315 gene expression changed statistically significantly following 50 µg/mL propolis treatment at all-time intervals. GeneSpring pathway analysis revealed significant effects of propolis on pathways related to cell division, including cell cycle and Rb in cancer. Propolis led to downregulation of CDK1, a gene associated with the cell cycle, and expression of genes associated with DNA repair mechanisms, including BRCA1, BARD1, RAD51, and CHEK1.

Conclusion: The findings of the microarray assay have highlighted that propolis from Türkiye has the capacity to inhibit the cell division process at many points and modulate the DNA repair mechanism.

Keywords Triple-negative breast cancer · Propolis · MDA-MB-231 · Microarray · qRT-PCR

Citation: Seyhan, M. F., Timirci Kahraman, Ö., Şenkal, N., Kısakesen, H. İ., Eronat, A. P., Öztürk, T., Yanar, F., Yılmaz Aydoğan, H. & Öztürk, O. Transcriptomic profiling reveals cell cycle and DNA repair pathway modulation in triple-negative breast cancer cells exposed to polyphenol-rich propolis. Experimed 2026; 16(1): 51-60. DOI: 10.26650/10.26650/experimed.1823647

This work is licensed under Creative Commons Attribution-NonCommercial 4.0 International License.

© 2026. Seyhan, M. F., Timirci Kahraman, Ö., Şenkal, N., Kısakesen, H. İ., Eronat, A. P., Öztürk, T., Yanar, F., Yılmaz Aydoğan, H. & Öztürk, O.

Corresponding author: Oğuz Öztürk oguzozturk@istanbul.edu.tr

INTRODUCTION

Breast cancer has the highest incidence rate in developed nations among all cancer types (1). The breast cancer subtype associated with the highest mortality, triple-negative breast cancer (TNBC), accounts for 15%–20% of all breast cancers. TNBC is characterized by the lack of hormone receptors and human epidermal growth factor receptor 2 (HER-2) (2, 3) together with high cellular invasiveness and metastatic capability (4). There are only restricted treatment approaches available for TNBC, such as chemotherapy, radiotherapy, surgery, and immunotherapy (2). Despite the noteworthy advancements in treatment approaches, poor prognosis of the disease, tumor heterogeneity, and metastatic capability, as well as the development of resistance to chemotherapeutic agents during treatment, significantly limit treatment success. Surgical intervention may not be sufficient to eradicate all cancerous cells, as these may have spread to other tissues. Moreover, use of radiotherapy and chemotherapeutics is often limited due to toxicity-related side effects. Immunotherapy, a novel and promising approach, can only be applied to suitable patients (5). Therefore, there is an evident requirement for developing therapeutic approaches that intensify existing treatment strategies for TNBC without side effects.

Propolis, a unique mixture of polyphenols and beeswax, is produced by bees to seal the hives (6); however, the chemical content is based on geographical and botanical variables such as climate, available plants, and time of collection (7). The extant literature suggests that propolis has diverse characteristics, including antibacterial, antioxidant, antifungal, anticancer, and cytotoxic effects (8). Therefore, propolis is also regarded as a promising antineoplastic drug owing to its components (9). Research has demonstrated that polyphenols function as chemopreventive compounds by modulating the activity of various signaling pathways including apoptosis. Moreover, mounting evidence from *in vitro* and *in vivo* investigations demonstrated their conventional treatment enhancing efficiency in various malignancies (9). Anticancer activity of propolis has been investigated, and several studies have shown that propolis inhibits cell proliferation, induces apoptosis and autophagy, and has antimetastatic activity (10, 11).

Previously, we have demonstrated the impacts of Turkish propolis on breast cancer cell lines (MCF-7, SK-BR-3, MDA-MB-231) and fibrocystic breast epithelial cell lines (MCF-10A) (11). In that study, have shown that propolis from Türkiye suppresses the viability of MDA-MB-231 cells at concentrations starting at 50 µg/mL after 48 and 72-hours without any cytotoxicity against MCF-10A cells. Moreover, the sample was noted to comprise the presence of the phenolic compounds krisin, galangin, pinostrobin, pinocembrin, pinobanksin, caffeic acid, apigenin, cinnamic acid, ferulic acid, kaempferol, coumaric acid, quercetin dihydrate, and luteolin using an high performance liquid chromatography (HPLC) system (11). Turkish propolis has been shown to have anticancer effects on all breast cancer cell lines and no similar effect on fibrocystic breast epithelial cell lines. Due to these impactful results, we wanted to investigate the anti-cancer effect of Turkish propolis whole-genome expression levels to elucidate molecular targets. The aim of this study was to characterize the global transcriptomic response of triple-negative breast cancer cells (MDA-MB-231) to treatment with a polyphenol-rich propolis extract from Türkiye, with a particular focus on identifying affected biological processes and signaling pathways using whole-genome expression profiling for the first time.

MATERIALS AND METHODS

Propolis Sample

Propolis (poplar-type) samples were collected from the Asian side of İstanbul. Biochemical and microbiological analyses conducted by Altıparmak Inc. (İstanbul, Türkiye) revealed no traces of heavy metals, pathogens, pesticides, or antibiotics (11). 4 mg/mL propolis was measured and solved in 60% ethanol/water (v/v) solution, and then ethanol was evaporated. Afterward, the propolis sample was filtered in 0.22 µm for sterilization. Then, the propolis sample was diluted 80-fold in cell culture medium to reach 50 µg/mL concentration.

Cell Culture

The MDA-MB-231 human breast cancer cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). Dulbecco's modified Eagle medium/F-12 (DMEM/F-12) containing 100 U/mL of penicillin and 10% fetal bovine serum (FBS) was used in the culturing process, which was conducted at 37°C with 5% CO_2 in incubator. All cell culture materials were obtained from Biochrome (Berlin, Germany).

Microarray Assay

Previously, we reported that the propolis extract (1-750 µg/mL) from İstanbul, Türkiye was exposed to breast cancer cell lines (MCF-7, SK-BR-3, MDA-MB-231) and fibrocystic breast epithelial cell line (MCF-10A) for 24, 48, and 72-hours. In MDA-MB-231 cells, it has been detected that propolis did not show cytotoxic effect. Expect the highest concentration as 750 µg/mL at 24-hour exposure. However, propolis has demonstrated cytotoxic effect starting from 50 µg/mL concentration at 48- and 72-hour exposure in MDA-MB-231 cells ($p<0.0001$) (11). According to the WST-1 cell proliferation assay, 50 µg/mL propolis concentrations for 48- and 72-hour treatment were selected for this study. For microarray assay, MDA-MB-231 cells were seeded in T75 flasks with the density of $1x10^6$ cell/flask in three biological triplicates and allowed to adhere at 37°C with 5% CO_2 overnight. Afterward, the medium was replaced with fresh medium supplemented with 3% FBS and incubated with a 50 µg/mL propolis solution for 48 and 72 hours, along with their untreated controls. Subsequently, total RNA was separated using the RNeasy Mini Kit (Qiagen, USA) Bioanalyzer 2100 (Agilent, CA, USA), used for

Table 1. Gene expression profiles in MDA-MB-231 cell line

		p < 0.05			p < 0.01		
		Upregulated	Downregulated	Total	Upregulated	Downregulated	Total
MDA-MB-231	0-48 h	20	55	75	5	18	23
	0-72 h	60	180	240	14	66	80

the determination of RNA integrity. For further experiments, only RNA samples with an RNA integrity number (RIN) higher than 7 were employed. Gene expression profiles were evaluated using a single-color scanning configuration for the Human Gene Expression Microarray (Agilent, CA, USA), 8x60K (ver. 2.0) array slides (Agilent, CA, USA). Bioinformatics and enrichment analysis were performed using GeneSpring software (GeneSpring 6.1, Silicon Genetics, Redwood City, CA, USA). The quantile normalization approach was then used to normalize the gene expression data.

Validation of Microarray Data Via Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Biological triplicate samples were obtained from cells and incubated with a 50 µg/mL propolis solution for 48 and 72-hours, respectively, and their untreated controls were used for qRT-PCR applications. The Custom RT2 Profiler PCR Array (Qiagen, SABiosciences, USA) was used to build forward and reverse primers. The RT2 HT First Strand Kit (Qiagen, SABiosciences, USA) was used to reverse-transcribe the total RNA, which was then processed through a Strategene Mx3005P System using the RT2 SYBR Green / ROX qPCR Master Mix (Qiagen, SABiosciences, USA). The relative differences in expression across groups were ascertained using the threshold cycle (CT) values of qRT-PCR . Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was selected as the housekeeping gene. Quantification was used to standardize each sample based on GAPDH. Due to the microarray data, the most statistically significantly changed genes were related to cell cycle and apoptosis. Therefore, the selected genes with particular primers and probes from Qiagen library are cyclin B1 (CCBB1) (NM_031966, RT2 Catalog number: PPH00126), cyclin B2 (CCBB2) (NM_004701, RT2 Catalog number: PPH00937), cyclin-dependent kinase 1 (CDK1) (NM_001786, RT2 Catalog number: PPH00116), Baculoviral IAP Repeat Containing 5 (BIRC5) (NM_001168, RT2 Catalog number: PPH00271), and Cell Division Cycle 25C (CDC25C) (NM_001790, RT2 Catalog number: PPH02198). Results were analyzed using RT² Profiler PCR Data Analysis version 3.5.

Statistical Analyses

Normalization of the samples was conducted using quantile normalization for microarrays. The analysis of microarray data groups was conducted utilizing the moderated t-test, Bonferroni FWER correction, and Gene Spring software (GeneSpring 6.1, Silicon Genetics, Redwood City, CA, USA). Those with a coefficient of variation (CV) of >50% were excluded. Following a moderated t-test and Bonferroni FWER correction, changes greater than 2-fold and findings with a p value of <0.05 and p value <0.01 were included in the analysis. Furthermore, logFC (log fold change values of changing genes) GO pathway enrichment analysis was performed using the Bioinformatics website (http://www.bioinformatics.com.cn/srplot).

RESULTS

Gene Expression Profiles

The microarray data revealed that 20 genes were detected as upregulated, 55 genes were downregulated at the 48th hour, 60 genes upregulated, and 180 genes downregulated at the 72nd hour ($p<0.05$) (Table 1). However, when $p<0.01$ was applied, 23 differentially expressed genes were identified at the 48th hour and 80 DEGs at the 72nd hour (Table 1). The biological functions and pathways related to DEGs in MDA-MB-231 cells treated with 50 µg/mL are presented in Tables 2 and 3, respectively. The pathway enrichment analysis using GeneSpring Software for DEGs revealed the following pathways: cell cycle, signal transduction, apoptosis modulation and signaling, G1-S cell cycle control, *Rb* in cancer, DNA damage response, cytokines and inflammatory response, integrated cancer pathways, mismatch repair, integrated breast cancer pathways, DNA replication, ATM signaling, tamoxifen metabolism, estrogen metabolism, and benzo(a)pyrene metabolism. Subsequently, the log-fold change (logFC) values of the modified genes were entered into the bioinformatics website platform to perform gene ontology (GO) pathway enrichment analysis, which indicated that 50 µg/mL propolis resulted in statistically significant downregulation of gene expression in pathways such as chromosome segregation, kinetochore organization, mitotic nuclear division, organelle fission, and spindle organization following 48 hours of exposure (Figure 1).

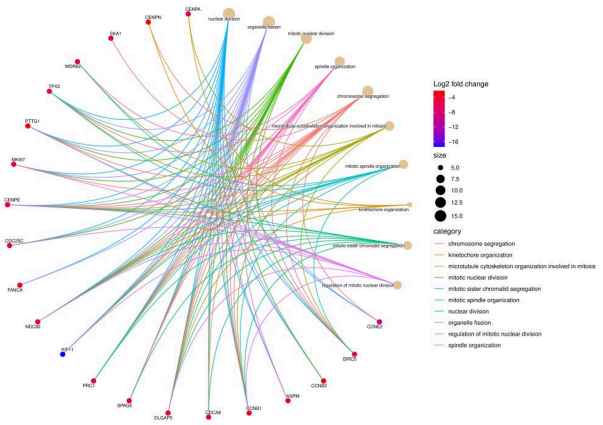

Figure 1. BPcnetplot image shows downregulated genes for 48 hours.

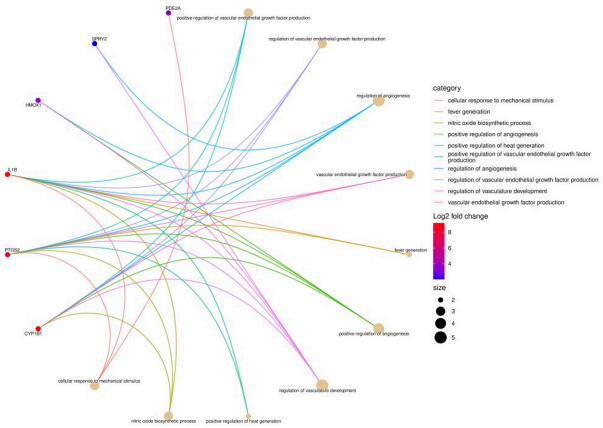

Figure 2. BPcnetplot image shows upregulated genes for 48 hours.

However, at the same time interval, propolis caused statistically significant upregulation of gene expression in the pathway including cellular response to mechanical stimulus, fever generation, nitric oxide biosynthetic process, positive regulation of angiogenesis, and positive regulation of vascular endothelial growth factor (VEGF) production (Figure 2). Interestingly, genes linked to chromosome segregation, mitotic sister chromatid separation spindle organization, organelle fission, DNA replication, and mitotic nuclear division were all altered in expression by 50 µg/mL dose of propolis, following a 72-hour exposure period (Figure 3). We have detected similar results after 72-hour exposure to propolis however, the statistically significantly changed number of downregulated genes related to cell cycle has been statistically significantly increased. In contrast, after 72-hours of propolis treatment, there was a statistically significant upregulation of gene expression in pathways such as the arachidonic acid metabolic process, estrogen metabolic process, fever generation, positive regulation of VEGF production, and unsaturated fatty acid metabolic process (Figure 4).

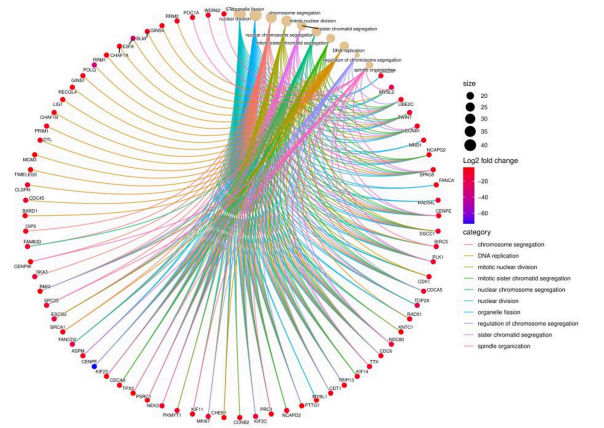

Figure 3. BPcnetplot image shows downregulated genes for 72-hours.

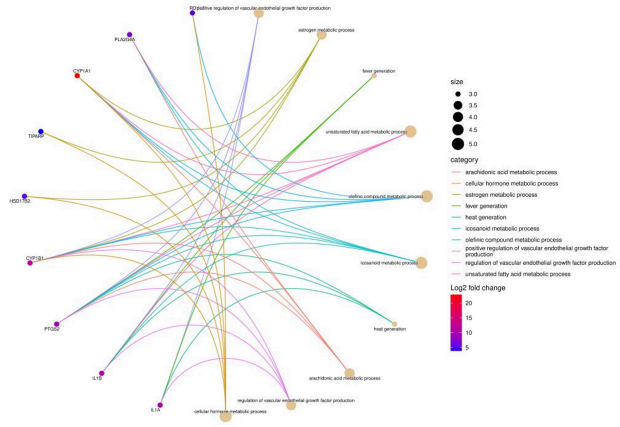

Figure 4. BPcnetplot image shows upregulated genes for 72-hours.

Table 2. Alterations in gene expression in regulatory pathways in MDA-MB-231 cells following treatment with 50 µg/mL of propolis after 48 hours

Gene Symbol	Genbank ID	Gene Name	p value	Log FC	Regulation
		Apoptosis Modulation and Signaling			
BIRC5	NM_001012271	baculoviral IAP repeat containing 5	0.0043	−2.333	down
		Cell Cycle			
CDC25C	NM_001790	cell division cycle 25 homolog C (S. pombe)	0.0220	−2.653	down
CCNE2	NM_057749	cyclin E2	0.0026	−3.241	down
CCNB1	NM_031966	cyclin B1	0.0194	−1.726	down
CCNB2	NM_004701	cyclin B2	0.0058	−1.841	down
MCM8	NM_182802	minichromosome maintenance complex component 8	0.0491	−1.112	down
PTTG1	NM_004219	pituitary tumor-transforming 1	0.0447	−1.172	down
		G1-S Cell Cycle Control			
CCNB1	NM_031966	cyclin B1	0.0194	−1.726	down
CCNE2	NM_057749	cyclin E2	0.0026	−3.241	down
		Rb in Cancer			
CCNB2	NM_004701	cyclin B2	0.0058	−1.841	down
CCNE2	NM_057749	cyclin E2	0.0026	−3.241	down
CCNB1	NM_031966	cyclin B1	0.0194	−1.726	down
		DNA Damage Response			
CCNB1	NM_031966	cyclin B1	0.0194	−1.726	down
CCNB2	NM_004701	cyclin B2	0.0058	−1.841	down
CCNE2	NM_057749	cyclin E2	0.0026	−3.241	down
CDC25C	NM_001790	cell division cycle 25 homolog C (S. pombe)	0.0220	−2.653	down
		Cytokines and Inflammatory Response			
IL1B	NM_000576	interleukin 1, beta	0.0097	3.136	up
		Integrated Cancer Pathway			
MMP1	NM_002421	matrix metallopeptidase 1 (interstitial collagenase)	4.11E-05	4.240	up
		Mismatch Repair			
LIG1	NM_000234	ligase I, DNA, ATP-dependent	0.0494	−1.877	down
		Tamoxifen Metabolism / Estrogen Metabolism / Benzo(a)pyrene metabolism			
CYP1B1	NM_000104	cytochrome P450, family 1, subfamily B, polypeptide 1	0.0061	3.174	up

Table 3. Alterations in gene expression in regulatory pathways in MDA-MB-231 cells following treatment with 50 µg/mL of propolis after 72-hours

Gene Symbol	Genbank ID	Gene Name	p value	Log FC	Regulation
		Cell Cycle			
CCNB1	NM_031966	cyclin B1	0.0256	−2.453	down
CCNB2	NM_004701	cyclin B2	0.0088	−2.981	down
CDC6	NM_001254	cell division cycle 6	0.0020	−3.1003	down
CDC45L	NM_003504	cell division cycle 45	0.0013	−3.5904	down
E2F2	NM_004091	E2F transcription factor 2	0.0147	−4.5347	down
RBL1	NM_002895	retinoblastoma-like 1	0.0046	−1.5591	down
MCM3	NM_002388	minichromosome maintenance complex component 3	0.0223	−1.4858	down
PLK1	NM_005030	polo-like kinase 1	0.0445	−3.5052	down
PKMYT1	NM_182687	protein kinase, membrane associated tyrosine/ threonine 1	0.0201	−3.2211	down
CHEK1	NM_001114121	checkpoint kinase 1	0.0443	−1.5908	down
PTTG1	NM_004219	pituitary tumor-transforming 1	0.0325	−1.4280	down

Table 3 continued

		G1-S Cell Cycle Control			
CCNB1	NM_031966	cyclin B1	0.0256	−2.453	down
CDKN2C	NM_078626	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	0.0182	−2.250	down
RBL1	NM_002895	retinoblastoma-like 1	0.0046	−1.559	down
E2F2	NM_004091	E2F transcription factor 2	0.0147	−4.534	down
CDK1	NM_001786	cyclin-dependent kinase 1	0.0130	−3.417	down
CDC45L	NM_003504	cell division cycle 45	0.0013	−3.590	down
MCM3	NM_002388	minichromosome maintenance complex component 3	0.0223	−1.485	down
PRIM1	NM_000946	primase, DNA, polypeptide 1 (49kDa)	0.0031	−3.132	down
		Rb in Cancer			
E2F2	NM_004091	E2F transcription factor 2	0.0147	−4.534	down
BARD1	NM_000465	BRCA1 associated RING domain 1	0.0186	−2.256	down
TOP2A	NM_001067	topoisomerase (DNA) II alpha 170kDa	0.0089	−4.411	down
CCNB2	NM_004701	cyclin B2	0.0088	−2.981	down
CCNB1	NM_031966	cyclin B1	0.0256	−2.453	down
TTK	NM_003318	TTK protein kinase	0.0040	−3.180	down
CDT1	NM_030928	chromatin licensing and DNA replication factor 1	0.0477	−2.258	down
CDK1	NM_001786	cyclin-dependent kinase 1	0.0130	−3.417	down
MCM3	NM_002388	minichromosome maintenance complex component 3	0.0223	−1.485	down
CDC45	NM_003504	cell division cycle 45	0.0013	−3.590	down
CHEK1	NM_001114121	checkpoint kinase 1	0.0443	−1.590	down
PRIM1	NM_000946	primase, DNA, polypeptide 1 (49kDa)	0.0031	−3.132	down
TYMS	NM_001071	thymidylate synthetase	2.74E-04	−3.731	down
		DNA Damage Response			
CCNB1	NM_031966	cyclin B1	0.0256	−2.4530	down
CCNB2	NM_004701	cyclin B2	0.0088	−2.9816	down
FANCD2	NM_001018115	Fanconi anemia, complementation group D2	1.56E-04	−4.009	down
BRCA1	NM_007300	breast cancer 1, early onset	0.0062	−2.364	down
RAD51	NM_002875	RAD51 recombinase	0.0171	−2.715	down
CHEK1	NM_001114121	checkpoint kinase 1	0.0443	−1.590	down
		Integrated Cancer Pathway			
BRCA1	NM_007300	breast cancer 1, early onset	0.0062	−2.364	down
MMP1	NM_002421	matrix metallopeptidase 1 (interstitial collagenase)	3.35E-05	5.048	up
BARD1	NM_000465	BRCA1 associated RING domain 1	0.01860	−2.256	down
BLM	NM_000057	Bloom syndrome, RecQ helicase-like	0.0016	−2.6615	down
PLK1	NM_005030	polo-like kinase 1	0.0445	−3.505	down
		Integrated Breast Cancer Pathway			
HIPK2	NM_001113239	homeodomain interacting protein kinase 2	0.0197	1.672	up
BRCA1	NM_007300	breast cancer 1, early onset	0.0062	−2.364	down
BLM	NM_000057	Bloom syndrome, RecQ helicase-like	0.0016	−2.661	down
RAD51	NM_002875	RAD51 recombinase	0.0171	−2.715	down
PLK1	NM_005030	polo-like kinase 1	0.0445	−3.505	down
MMP1	NM_002421	matrix metallopeptidase 1 (interstitial collagenase)	3.35E-05	5.048	up
BARD1	NM_000465	BRCA1 associated RING domain 1	0.0186	−2.256	down

Table 3 continued

		Integrin Mediated Cell Adhesion			
CAV1	NM_001753	caveolin 1, caveolae protein, 22kDa	0.0054	−2.358	down
ITGAE	NM_002208	integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide)	0.0473	−1.246	down
ITGB3	NM_000212	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	0.0234	1.621	up
		EGF-EGFR Signaling			
CAV1	NM_001753	caveolin 1, caveolae protein, 22kDa	0.0054	−2.358	down
CBLB	NM_170662	Cbl proto-oncogene B, E3 ubiquitin protein ligase	0.0311	1.352	up
MYBL2	NM_002466	v-myb avian myeloblastosis viral oncogene homolog-like 2	0.0106	−4.407	down
PLCE1	NM_016341	phospholipase C, epsilon 1	0.0413	−2.713	down
		DNA Replication			
CDC45L	NM_003504	cell division cycle 45	0.0013	−3.590	down
CDC6	NM_001254	cell division cycle 6	0.0020	−3.100	down
CDT1	NM_030928	chromatin licensing and DNA replication factor 1	0.0477	−2.258	down
PRIM1	NM_000946	primase, DNA, polypeptide 1 (49kDa)	0.0031	−3.132	down
MCM3	NM_002388	minichromosome maintenance complex component 3	0.0223	−1.485	down
		ATM Signaling			
BRCA1	NM_007300	breast cancer 1, early onset	0.0062	−2.364	down
FANCD2	NM_001018115	Fanconi anemia, complementation group D2	1.56E-04	−4.009	down
		Tamoxifen Metabolism / Estrogen Metabolism / Benzo(a)pyrene metabolism			
CYP1A1	NM_000499	cytochrome P450, family 1, subfamily A, polypeptide 1	0.0082	4.511	up
CYP1B1	NM_000104	cytochrome P450, family 1, subfamily B, polypeptide 1	0.0024	3.530	up
		Cytokines and Inflammatory Response			
IL1A	NM_000575	interleukin 1, alpha	0.0296	3.360	up

Validation with qRT-PCR for Selected Genes from Microarray Analysis

qRT-PCR tests were utilized to verify the expressional changes in DEGs related to cell cycle and apoptosis. At the 48th hour of exposure, 4 genes at (*CCNB1*, *CCNB2*, *CDC25C*, and *BIRC5*) and 3 genes at the 72nd hour (*CCNB1*, *CCNB2*, *CDK1*) were tested. As demonstrated in Tables 4 and 5, qRT-PCR data demonstrated a high degree of correlation with data obtained from the microarray analysis.

Table 4. Fold changes of selected DEGs in microarray and qRT-PCR at 48th hour (MDA-MB-231)

Gene Symbol	Gene Description	Microarray for 48 h	qRT-PCR for 48 h
CCNB1	cyclin B1	−1.72	−2.11
CCNB2	cyclin B2	−1.84	−1.52
CDC25C	cell division cycle 25 homolog C (S. pombe)	−2.33	−3.12
BIRC5	baculoviral IAP repeat containing 5	−2.65	−3.06

DEG: Differentially expressed genes, IAP: Inhibitor of apoptosis protein.

Table 5. Fold changes of selected DEGs in microarray and qRT-PCR at 72nd hour (MDA-MB-231)

Gene Symbol	Gene Description	Microarray for 72 h	qRT-PCR for 72 h
CCNB1	cyclin B1	−2.45	−3.81
CCNB2	cyclin B2	−2.98	−3.54
CDK1	cyclin-dependent kinase 1	−3.41	−4.75

DEG: Differentially expressed genes.

DISCUSSION

TNBC is characterized by the lack of target receptors and reduced responsiveness to treatment (12, 13). Considerable work has been done to group TNBC subtypes into discrete molecular clusters according to their distinct signaling traits, which will allow the use of various targeted therapeutics (14). Until recently, patients with TNBC subtypes had very limited treatment options due to the absence of an effective targeted drug.

Propolis demonstrated its ability to influence diverse biological processes, such as cell division, initiation of proapoptotic pathways, and metastasis, together with regulation of angiogenesis (7, 15). Recently, various studies have noted the chemosensitizing effects of propolis components on cytostatics, as well as alterations of the tumor microenvironment. Furthermore, polyphenols have been demonstrated to be highly effective in preventing the cytotoxic adverse effects of anticancer drugs by inducing apoptosis in normal cells or interrupting the cell cycle (16). Moreover, a number of *in vitro* experiments demonstrated that both propolis extracts and their active components exert antiviability effects on different breast cancer cell lines (17, 18), colon cancer (19), cervical cancer, and lung cancer (20). In addition, bee pollen and water-soluble propolis showed strong antiproliferative effects on hormone receptor-positive breast cancer (MCF-7) cells (21). Polyphenols present in propolis have been demonstrated to influence multiple signaling pathways in TNBC and proposed as potential targets against TNBC (22). Among these, a significant component of Brazilian propolis, artepillin C, has been shown to possess antitumor effects in ER+/PR+ and TNBC cells (23). Furthermore, the combination of pinostrobin, pinocembrin, and pinobanksin exhibited apoptotic-induced cytotoxic effects on MDA-MB-231 cells (24).

Numerous polyphenols, including apigenin, quercetin, hesperetin, kaempferol, and resveratrol, have been shown to trigger multiple mechanisms in TNBC, including cell cycle arrest, apoptosis induction, autophagy, proliferation inhibition, invasion inhibition, and migration (21). Propolis rich with polyphenols obtained from Türkiye altered 11 pathways, including apoptosis and cell division. Microarray analysis revealed that DEGs are associated with intracellular pathways, such as cell cycle, cell cycle control, DNA damage control, cell adhesion, DNA replication, ATM signaling, tamoxifen metabolism, estrogen metabolism, and benzo(a)pyrene metabolism, and all of these are related to breast cancer development and progression (Tables 2 and 3). Furthermore, the selected cell cycle-related gene expression data were validated via qRT-PCR analysis.

In the present study, 50 µg/mL propolis administered to MDA-MB-231 following a 72-hour treatment period yielded the most statistically significant results based on the whole-genome expression data. A pathway analysis was conducted utilizing GeneSpring Software, which revealed significant alterations in pathways associated with cell division, including the cell cycle, DNA damage response, and integrated breast cancer pathway (Table 3). These findings show that propolis halts cell division via checkpoints and mechanisms. Notably, the findings of this study indicate that propolis treatment inhibits *CDK1*, given the established association between abnormal *CDK* activation and carcinogenesis (25). Normally, the *MCM3* gene, which is associated with the cell cycle, is overexpressed in breast cancer (26); however, we have detected that propolis treatment led to the downregulation of the *MCM3* gene. Furthermore, we have found similar results that Turkish propolis inhibited cell division by targeting multiple genes in SK-BR-3 cells (27). Interestingly, a polyphenolic mixture containing seven polyphenols inhibited the cell cycle by causing the downregulation of several genes (28). Moreover, various mutations in the DNA repair mechanism can cause genomic instability and carcinogenesis (29). The present study also demonstrated significant downregulation of genes related to DNA repair, particularly *BRCA1*, *BARD1*, *RAD51*, *FANCD2*, and *CHEK1*, which play important roles (30). In addition to pathways associated with cell cycle and DNA repair, the enrichment of gene ontology analysis showed the positive regulation of VEGF production, a key mediator of angiogenesis. In cancer biology, elevated VEGF expression has traditionally been associated with enhanced tumor

angiogenesis, metastasis, and poor prognosis, particularly in highly vascularized malignancies (31). However, upregulation of VEGF-related genes in response to a treatment does not necessarily indicate a straightforward pro-tumor effect. In transcriptomic studies of complex natural products, VEGF signaling can reflect context-dependent or adaptive cellular responses rather than direct promotion of angiogenesis. For example, stress responses such as hypoxia-inducible factor (HIF) activation, oxidative stress, and metabolic perturbations can induce VEGF expression as part of an adaptive attempt to restore homeostasis (32). Taken together, these observations suggest that the upregulation of VEGF-associated genes in our study may reflect an early compensatory response of MDA-MB-231 cells to propolis-induced cellular perturbations rather than unequivocal stimulation of angiogenesis.

This study has certain limitations. Initially, the study was conducted using a single TNBC cell line (MDA-MB-231). The findings may not fully represent responses across different TNBC subtypes or breast cancer models. Validation of these results in additional TNBC cell lines and, where appropriate, non-malignant breast epithelial cells would strengthen the generalizability of the conclusions. Also, the study is entirely cell-culture-based and lacks tumor microenvironment crosstalk. In further studies, we plan *in vivo* experiments to clarify the anticancer effect of propolis more comprehensively. Finally, although selected gene expression changes were validated by qRT-PCR, the study primarily focuses on transcriptomic alterations. Changes at the mRNA level do not always correspond to functional protein activity or phenotypic outcomes. Additional functional assays for protein expression should be added in future studies.

CONCLUSIONS

This study, using microarray assay, revealed that Turkish propolis, previously found to demonstrate anticancer effects by decreasing cell proliferation and triggering apoptosis in TNBC cells, exhibits the aforementioned effects through changes in multiple genes associated with intracellular pathways related to TNBC development. In view of the absence of targeted drugs for TNBC, its high degree of aggressiveness and poor prognosis, and the need for effective treatment options, polyphenols, due to their widespread availability, affordability, and safety, represent a promising class of compounds for further investigation in TNBC treatment and prevention.

Acknowledgements The propolis sample was obtained and analyzed by Altıparmak Inc., for which the authors are grateful

Ethics Committee Approval Since commercial cell lines were used in this study, ethical approval was not required.

Peer Review Externally peer-reviewed.

Author Contributions Conception/Design of Study: O.Ö., H.Y.A., T.Ö. Data Acquisition: M.F.S., Ö.T.K., A.P.E. Data Analysis Interpretation: M.F.S., N.Ş., H.İ.K., F.Y. Drafting Manuscript: M.F.S., Ö.T.K., A.P.E., N.Ş., H.İ.K., F.Y.

Conflict of Interest The authors have no conflict of interest to declare.

Financial Disclosure This research's design is a crucial component of a cooperative research process funded by a grant and the Istanbul Development Agency (ISTKA/2010/KBO-62).

Author Details

Mehmet Fatih Seyhan
[1] İstanbul Yeni Yüzyıl University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, İstanbul, Türkiye
0000-0002-6218-0049

Özlem Timirci Kahraman
[2] İstanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Molecular Medicine, İstanbul, Türkiye
0000-0002-2641-5613

Naci Şenkal
[3] İstanbul, University, Faculty of Medicine, Department of Internal Medicine, İstanbul, Türkiye
0000-0001-7072-8724

Halil İbrahim Kısakesen
[4] İstanbul, Technical University, Faculty of Science, Department of Molecular Biology and Genetics, İstanbul, Türkiye
0000-0002-0351-4205

Allison Pınar Eronat
[5] Halic University, Department of Molecular Biology and Genetics, İstanbul, Türkiye
0000-0002-8095-1759

Tülin Öztürk
[6] İstanbul University-Cerrahpasa, Faculty of Medicine, Department of Pathology, İstanbul, Türkiye
0000-0001-6910-1131

Fatih Yanar
[7] Boğazici University, Department of Molecular Biology and Genetics, İstanbul, Türkiye
0000-0003-1399-3382

Hülya Yılmaz Aydoğan
[2] İstanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Molecular Medicine, İstanbul, Türkiye
0000-0002-8837-6664

Oğuz Öztürk
[2] İstanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Molecular Medicine, İstanbul, Türkiye
0000-0002-2439-9269 oguzozturk@istanbul.edu.tr

REFERENCES

1. Wilkinson L, Gathani T. Understanding breast cancer as a global health concern. Br J Radiol 2022; 95(1130): 20211033.
2. Obidiro O, Battogtokh G, Akala EO. Triple negative breast cancer treatment options and limitations: future outlook. Pharmaceutics 2023; 15(7): 1796.

3. Zagami P, Carey LA. Triple negative breast cancer: Pitfalls and progress. NPJ Breast Cancer 2022; 8(1): 95.
4. Vagia E, Mahalingam D, Cristofanilli M. The landscape of targeted therapies in TNBC. Cancers 2020; 12(4): 916.
5. Keihan Shokooh M, Emami F, Jeong JH, Yook S. Bio-inspired and smart nanoparticles for triple negative breast cancer microenvironment. Pharmaceutics 2021;13(2): 287.
6. Simone-Finstrom M, Spivak M. Propolis and bee health: The natural history and significance of resin use by honey bees. Apidologie 2010; 41(3): 295-311.
7. Stojanović S, Najman SJ, Bogdanova-Popov B, Najman SS. Propolis: chemical composition, biological and pharmacological activity-A review. Acta Med Medianae 2020; 59(2): 108-13.
8. Anjum SI, Ullah A, Khan KA, Attaullah M, Khan H, Ali H, et al. Composition and functional properties of propolis (bee glue): A review. Saudi J Biol Sci 2019; 26(7): 1695-703.
9. Oršolić N, Jazvinšćak Jembrek M. Molecular and cellular mechanisms of propolis and its polyphenolic compounds against cancer. Int J Mol Sci 2022; 23(18): 10479.
10. Valivand N, Aravand S, Lotfi H, Esfahani AJ, Ahmadpour-Yazdi H, Gheibi N. Propolis: a natural compound with potential as an adjuvant in cancer therapy – a review of signaling pathways. Mol Biol Rep 2024; 51(1): 931.
11. Seyhan MF, Yılmaz E, Timirci-Kahraman Ö, Saygılı N, Kısakesen Hİ, Gazioğlu S, et al. Different propolis samples, phenolic content, and breast cancer cell lines: Variable cytotoxicity ranging from ineffective to potent. IUBMB Life 2019; 71(5): 619-31.
12. do Nascimento RG, Otoni KM. Histological and molecular classification of breast cancer. Mastology 2020; 30: e20200024.
13. Dewi C, Fristiohady A, Amalia R, Khairul Ikram NK, Ibrahim S, Muchtaridi M. Signaling pathways and natural compounds in triple-negative breast cancer cell line. Molecules 2022; 27(12): 3661.
14. Wang DY, Jiang Z, Ben-David Y, Woodgett JR, Zacksenhaus E. Molecular stratification within triple-negative breast cancer subtypes. Sci Rep 2019; 9(1): 19107.
15. Popova M, Giannopoulou E, Skalicka-Woźniak K, Graikou K, Widelski J, Bankova V, et al. Characterization and biological evaluation of propolis from Poland. Molecules 2017; 22(7): 1159.
16. Noureddine H, Hage-Sleiman R, Wehbi B, Fayyad-Kazan H, Hayar S, Traboulssi M, et al. Chemical characterization and cytotoxic activity evaluation of Lebanese propolis. Biomed Pharmacother 2017; 95: 298-307.
17. Jung BI, Kim MS, Kim HA, Kim D, Yang J, Her S, et al. Caffeic acid phenethyl ester, a component of beehive propolis, is a novel selective estrogen receptor modulator. Phytother Res 2010; 24(2): 295-300.
18. Vatansever SH, Sorkun K, Ismet Deliloğlu Gurhan S, Ozdal-Kurt F, Turkoz E, Gencay O, et al. Propolis from Turkey induces apoptosis through activating caspases in human breast carcinoma cell lines. Acta Histochem 2010;112(6): 546-56.
19. Ishihara M, Naoi K, Hashita M, Itoh Y, Suzui M. Growth inhibitory activity of ethanol extracts of Chinese and Brazilian propolis in four human colon carcinoma cell lines. Oncol Rep 2009; 22(2): 349-54.
20. Li F, Awale S, Tezuka Y, Kadota S. Cytotoxicity of constituents from Mexican propolis against a panel of six different cancer cell lines. Nat Prod Commun 2010; 5(10):1601-06.
21. Amalia E, Diantini A, Subarnas A. Water-soluble propolis and bee pollen of Trigona spp. from South Sulawesi Indonesia induce apoptosis in the human breast cancer MCF-7 cell line. Oncol Lett 2020; 20(5): 274.
22. Farghadani R, Naidu R. The anticancer mechanism of action of selected polyphenols in triple-negative breast cancer (TNBC). Biomed Pharmacother. 2023; 165: 115170.
23. de Freitas Meirelles LE, de Assis Carvalho ARB, Ferreira Damke GMZ, Souza RP, Damke E, de Souza Bonfim-Mendonça P, et al. Antitumoral potential of Artepillin C, a compound derived from Brazilian propolis, against breast cancer cell lines. Anticancer Agents Med Chem 2024; 24(2): 117-24.
24. Melekoglu A, Ceviz AB, Eronat AP, Ozturk T, Pehlivan F, Yılmaz-Aydogan H, et al. Synergistic and dose-dependent effects of pinostrobin, pinocembrin and pinobanksin on different breast cancer cell lines. Kafkas Univ Vet Fak Derg 2024; 30 (1): 23-30.
25. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nature Reviews Cancer 2009; 9(3): 153- 66.
26. Zhao Y, Wang Y, Zhu F, Zhang J, Ma X, Zhang D. Gene expression profiling revealed MCM3 to be a better marker than Ki67 in prognosis of invasive ductal breast carcinoma patients. Clin Exp Med 2020; 20(2): 249-59.
27. Seyhan MF, Timirci-Kahraman Ö, Eronat AP, Yılmaz E, Ceviz AB, Kisakesen HI, et al. Whole genome expression analysis identifies multiple targeted integrative effects of polyphenol-rich propolis on HER-2-positive breast cancer cell line. Rec Nat Prod 2024; 18(2): 201-11.
28. Seyhan MF, Eronat AP, Diren A, Tuzuner MB, Ceviz AB, Yilmaz-Aydogan H, et al. A mixture of potent polyphenolic anticarcinogens: Microarray analysis of their efficacy in MCF-7 and MCF-10A cell lines. Rec Nat Prod 2026; 20(4): e25103658.
29. Tufail, M. DNA repair pathways in breast cancer: from mechanisms to clinical applications. Breast Cancer Res Treat 2023; 200(3): 305-21.
30. Woditschka S, Evans L, Duchnowska R, Reed LT, Palmieri D, Qian Y, et al. DNA double-strand break repair genes and oxidative damage in brain metastasis of breast cancer. J Natl Cancer Inst 2014; 106(7): dju145.
31. Shi Z, Kuai M, Li B, Akowuah CF, Wang Z, Pan Y, Tang M, Yang X, Lü P. The role of VEGF in cancer angiogenesis and tumorigenesis: Insights for anti-VEGF therapy. Cytokine 2025; 189: 156908.
32. Liu X, Zhang J, Yi T, Li H, Tang X, Liu D, Wu D, Li Y. Decoding tumor angiogenesis: pathways, mechanisms, and future directions in anti-cancer strategies. Biomark Res 2025; 13(1): 62.