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# Whole Genome Expression Analysis Identifies Multiple Targeted Integrative Effects of Polyphenol-Rich Propolis on HER-2-Positive Breast Cancer Cell Line

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**Abstract:** Natural products have been focused by researchers due to their important anticarcinogenic characteristics in the treatment of cancer with the slightest side effects possible. Propolis is one of the most prominent candidates among these natural products in terms of its anticancer features. In this study we aim to research the effects of Anatolian propolis on ER/PR-, HER-2/neu+ human breast cancer cell line SK-BR-3 with intent to clarify the molecular mechanism propolis in HER+ breast cancers in overview of whole genomic expression for the first time via a microarray experiment. Afterwards, microarray data was validated via real time PCR with the selected genes. After performing bioinformatic analysis via GeneSpring Software and String analysis, a 50 µg/mL dose of propolis affected several pathways of HER-2 positive breast cancer cells including cell cycle, DNA repair and apoptosis especially at 48th hour exposure. In contrast, after exposure to 50 µg/mL dose of propolis, up-regulated genes were detected at diverse pathways such as immune response, cell migration regulation, organization of cell-cell adhesion, etc. For this reason, we proposed that polyphenol-rich propolis can be used in the treatment of HER-2 positive breast cancer with characteristics of less toxic than the current treatment methods.

**Keywords:** Breast cancer; HER-2; microarray; flavonoids; bioinformatics. 

## 1. Introduction

Breast cancer is the most widely diagnosed cancer in Western women and second after lung cancer as a reason of cancer-associated death [1]. Although the mortality rates have begun to diminish due to escalated utilization of mammographic screening and supporting treatments, death rate of breast cancer is

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still second amongst cancer related deaths [2]. Breast cancer has different kinds of subtypes with various biological characteristics aiding us during treatment. There are important features in the standard taxonomy of and treatment of breast cancer, like tumor size, lymph node involvement, histological grade, patient's age and presence of estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER-2/neu) [3]. The treatment for breast cancer is comprised of the alliance of surgery, radiation therapy, hormone therapy and chemotherapy. In addition, for patients that are positive for HER-2/neu, estrogen and progesterone receptors targeted therapies are utilized [4].

Human epidermal growth factor receptor 2 (HER-2) is amplified and/or overexpressed in nearly 15-20% of breast cancers [5, 6]. Amplification or overexpression causes accretion of HER-2 in plasma membrane, resulting in constant activation of HER-2 intracellular signaling pathways, which consequently initiates cell proliferation, survival, motility and invasion in breast cancer cells [7]. Associated pathways with HER-2 responsiveness are mainly STAT, PI3K, MAPK and non-receptor tyrosine kinase Src. Therefore, HER-2-related over activation of these pathways are considered to possess key roles in metastasizing of HER-2 positive breast cancer subtype [8]. In clinics, the presence of HER-2 gene amplification/protein overexpression is described as an indicator of poor prognosis and a biomarker for the treatment with Trastuzumab/Herceptin which is a monoclonal antibody [9]. However, use of Trastuzumab in the therapy of HER-2-positive breast cancer patients face resistance mechanisms. Thus, clarifying the resistance mechanisms and the affiliation of novel agents may introduce improved inhibition of the HER family receptor signaling [10].

Propolis is a glue-like natural substance collected from leaves, trunk and buds of various plants and produced by honeybees in hives [11]. Context of propolis is highly variable due to wide spectrum of vegetation surrounding honeybees while gathering materials [12]. Propolis broadly comprises diverse compounds, such as phenolic acids or their esters, flavonoids, β-steroids, aromatic aldehydes, stilbenes, fatty acids, terpens, and alcohols [13]. Due to the variability of polyphenolic content of propolis, it needs to be standardized for much more beneficial effects as a potential agent against different diseases [14]. Propolis has a long history in folk medicine, having been used as a natural drug by physicians for the treatment of several diseases utilizing its antiseptic, antibacterial, anti-inflammatory and regenerative properties [15].

In our previous study, we have reported the anticarcinogenic effect of Anatolian propolis on MCF-7, MDA-MD-231, SK-BR-3 breast cancer cell line and MCF-10A fibrocystic breast epithelial cell line. Our findings showed that Anatolian propolis inhibited the proliferation of SKBR-3 cells starting from the dose of 50 µg/mL at $24^{th}$, $48^{th}$ and $72^{nd}$ hours of exposure [16]. On the contrary, Anatolian propolis did not display any cytotoxic effect on MCF-10A cells. Moreover, Anatolian propolis caused SK-BR-3 cells to undergo apoptosis at 50 to 250 µg/mL at 48th and 72nd hour based on Annexin V-PI assay findings. According to LC-MS/MS analysis data Anatolian propolis is rich in the content of caffeic acid, chrysin, p-OH benzoic acid, galangin, pyrogallol, fumaric acid, p-coumaric acid, pinostrobin, pinobanksin and pinocembrin [16]. It can be suggested that the anticarcinogenic effect of propolis is due to its polyphenolic content. In this study, because of galangin, pinostrobin, caffeic acid and kaempferol were found rich in the propolis in our previous research, we have selected these compounds in order to investigate their effect on SK-BR-3 cells by performing WST-1 cell proliferation assay. In addition, our goal was to look at the impacts of Anatolian propolis on ER/PR-, HER-2/neu+ human breast cancer cell line SK-BR-3 to clarify the molecular mechanism of propolis in HER+ breast cancers in overview of whole genomic expression for the first time.

## 2. Materials and Methods

### *2.1. Propolis extract*

Propolis was collected from Asian side of Istanbul, Türkiye by Altiparmak Inc. (Istanbul). Propolis extracts were dissolved in 60% ethanol and filtered in 0.22 µm for the purpose of sterilization. Altiparmak Apilab Laboratories (Istanbul, Türkiye) conducted all the analysis such as biochemical and microbiological and no heavy metals (cadmium, lead, mercury, tin) or arsenic were observed, as well as no pathogens, pesticides or antibiotics in the samples [16]. Based on the that study, 50 µg/mL dose of Anatolian propolis were determined as effective dose in terms of cell viability/cytotoxicity on SK-BR-3 cell line, therefore, this dose was used for microarray and real time PCR analyses [16].

*2.2. Cell Culture*

All cell culture substances were acquired from Biochrome (Berlin, Germany). ER/PR-, HER-2/neu+ human breast cancer cell line, SKBR-3, was purchased from ATCC (American Type Culture Collection). Cells were cultured in McCoy's 5A (Modified) medium (Biochrome) containing 10% Fetal bovine serum (FBS), 1% Glutamine and 1% Penicillin at 37°C in 5% $C0_2$.

*2.3. WST-1 Cell Proliferation Assay*

The Cell Proliferation Reagent WST-1 (Roche, Manheim, Germany) was used for the cell proliferation analysis. After cells were counted via Vi-Cell XR Cell Viability Analyzer (Beckman Coulter, USA), cultured in 96 well plates (Greiner Bio-one, Austria) at a density of $1x10^4$ cell/ well. The cells were allowed to adhere overnight, subsequently the medium was renewed with fresh medium added with 3% FBS. Then, following flavonoids were applied to the cells as 0.7, 1.4, 5, 10, 15, 20, 25, 30, 40 and 45 µg/mL of galangin; 0.05, 0.10, 1, 2.5, 5, 7.5, 10, 15, 20 and 30 µg/mL of caffeic acid; 0.7, 1.4, 5, 10, 15, 20, 25, 30, 40 and 45 µg/mL of pinostrobin; 0.5, 1, 2.5, 5, 10, 15, 25, 30, 40 and 50 µg/mL of kaempferol at 24, 48 and 72 hours. At selected time intervals, we applied 10 µl/well WST-1 and exposed them for approximately 4 hours at 37°C in 5% $CO_2$. Afterwards, we detected the impacts of WST-1 by quantifying absorbance at 450 nm with the reference wavelength set at 620 nm using Multiscan ELISA reader (Thermo Fisher Scientific, Germany). The data are presented as a relative proliferation index scale (mean ± SD) as percentages of absorbance values from control wells. The approximated sigmoidal curve was used to derive the IC50 values. Every test was run in four duplicates.

*2.4. RNA Isolation and Microarray Analysis*

Cells were cultured in T25 flask at the density of $1x10^6$ cells and incubated with 50µg/mL propolis. Following 24 and 48 hours of propolis treatment, a RNeasy Plus Mini Kit (Qiagen) was used for total RNA isolation process. Afterwards, RNA integrity was measured by using Bioanalyzer (Agilent); all RIN values results were > 7. Microarray analysis was conducted to examine the gene expression profiles. Overall alterations in gene expression were evaluated using Custom Gene Expression Microarray, 8x60K (Agilent, CA, USA), as recommended by the manufacturer. Overall changes in gene expression were examined with GeneSpring software (GeneSpring 13.0 Silicon Genetics, Redwood City, CA, USA). All tests were performed in quadruplicate.

*2.5. Reverse Transcriptase- PCR (RT-PCR) Analysis*

To confirm the microarray findings, the isolated total RNA, which was treated with different doses, was amplified using Brilliant III Ultra-Fast QRT-PCR Master Mix (Agilent, CA, USA) with specific primers and probes of selected genes such as E2F Transcription Factor 1 (E2F1), Cyclin-dependent kinase inhibitor 3 (CDKN3), cyclin B1 (CCNB1), cyclin B2 CCBB2, Cyclin-dependent kinase 1 (CDK1), Baculoviral IAP Repeat Containing 5 (BIRC5), BCL2 Apoptosis Regulator (BCL2), Cell Division Cycle 25C (CDC25C), BCL2 Associated Agonist Of Cell Death (BAD), TNF Superfamily Member 10 (TNFSF10), X-Linked Inhibitor Of Apoptosis (XIAP) and Baculoviral IAP Repeat Containing 3 (BIRC3).

*2.6. Statistical Analysis*

In statistical analysis of WST-1 assay, all values were expressed as mean ± standard deviation. Statistical evaluations were performed using Two-Way Anova and Tukey test and $p < 0.05$ value were determined as significant. GraphPad Prism 6 (GraphPad Prism Software, San Diego, CA, USA) was used for all statistical analysis. Raw data of the microarray study were normalized with Quantile Normalization and analyzed using the GeneSpring software version 13.0 (Silicon Genetics, Redwood City, CA, USA). The data with the coefficient of variation (CV) over 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 to the analysis. Altered gene expressions ($p<0.05$) were admitted to string-db.org. Interacting proteins with highest confidence were selected (0.900) and the disconnected nodes were discarded.

## 3. Results and Discussion

### *3.1. WST-1 Cell Proliferation Assay*

Cell viability/cytotoxicity effects of galangin, kaempferol, caffeic acid and pinostrobin were examined on SK-BR-3 cell line via WST-1 cell proliferation assay. SK-BR-3 cells were exposed to these compounds with a range of diverse doses at 24$^{th}$, 48$^{th}$ and 72$^{nd}$ hours. Cell viability/cytotoxicity effects of selected compounds on SK-BR-3 cells are given in Figure 1. Galangin showed cytotoxic effect after doses of 15 µg/mL at all time intervals, with IC50 values of 29.22 at the 24$^{th}$ hour, 27.52 at the 48$^{th}$ hour and 24.88 at the 72$^{nd}$ hour. The most cytotoxic effect of kaempferol was found at 24$^{th}$ exposure on SK-BR-3 with IC50 value of 7.578. IC50 values of kaempferol were found on SK-BR-3 cells much higher as 48$^{th}$ and 72$^{nd}$ hour, 36.49 and 35.29 respectively. Caffeic acid showed cytotoxic effect especially at 24$^{th}$ exposure on SK-BR-3 cell line (IC50 value 13.00) however, there is no similarly cytotoxic effect detected after long term exposure. IC50 value of caffeic acid were found as 80.57 at 48$^{th}$ hour exposure. Pinostrobin inhibited cell viability after the amount of 15 µg/mL at all time intervals, with IC50 values of 24.65 at the 24$^{th}$ hour, 22.69 at the 48$^{th}$ hour and 19.15 at the 72$^{nd}$ hour.

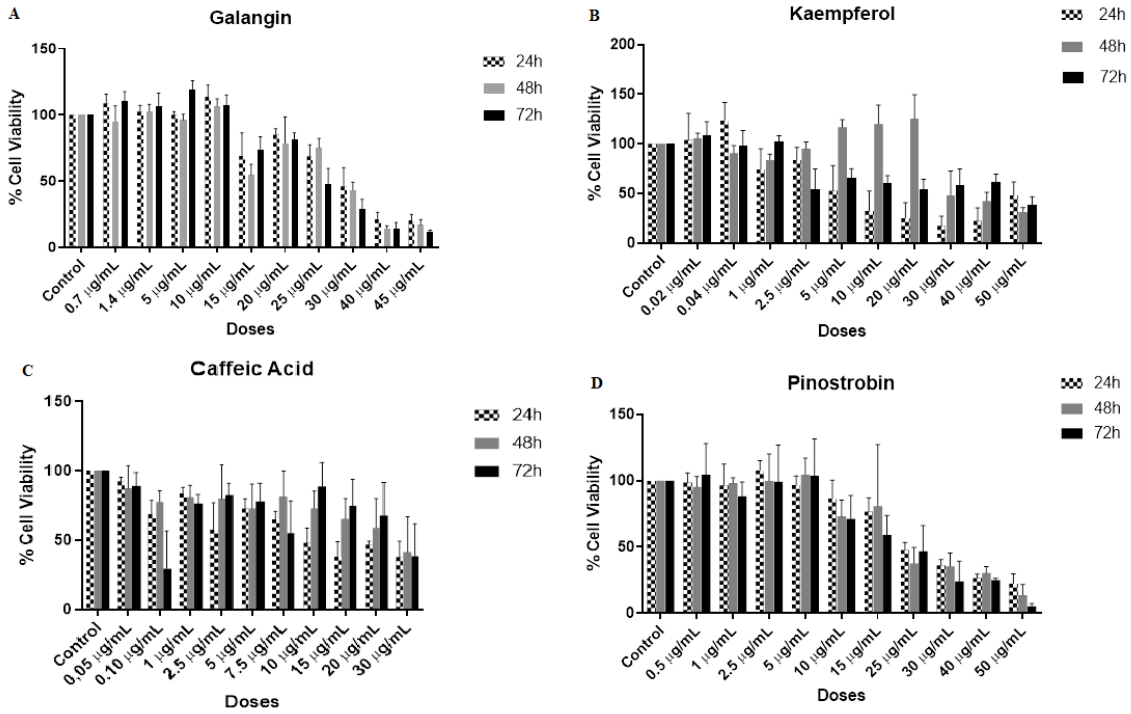


**Figure 1**. Effects of selected flavonoids on SKBR3 cell line

### *3.2 Microarray Analysis*

Both 24h and 48h data were compared with the control group. When compared to hour 0, 210 genes were differentially altered ($p < 0.05$) due to propolis on 24th hour in SK-BR-3, of which 123 were up regulated and 87 down regulated (Supplement Table 1). On the 48th hour, 637 genes were differentially regulated ($p < 0.05$), of which 265 were up regulated and 372 down regulated (Supplement Table 2). Among gene expressions that were statistically significantly altered, 142 were mutual (90 up-regulated and 52 down regulated). Moreover, when we determined a more restricted cutoff ($p < 0.01$), 50 genes were significantly regulated, among which 29 were up regulated and 21 down-regulated in SK-BR-3 at the 24th hour. At 48th hour, expressions of 248 genes were significantly altered, among which 91were up regulated and 157 down-regulated (Table 1). In light of bioinformatic analysis using GeneSpring software version 13.0 program and string analysis, we have summarized expression of the most important genes in Table 2 according to related pathways.

**Table 1.** Number of genes regulated by propolis in SK-BR-3 cell line

| | P < 0.05 | | | P < 0.01 | | |
|---|---|---|---|---|---|---|
| | **Up- regulated** | **Down- regulated** | **Total** | **Up- regulated** | **Down- regulated** | **Total** |
| **0-24 h** | 123 | 87 | 210 | 29 | 21 | 50 |
| **0-48 h** | 265 | 372 | 637 | 91 | 157 | 248 |

**Table 2.** Summary of Altered Genes by Propolis in Various Pathway

| | Gene Symbol | Gene Bank Accession no | Gene Name | P Value | Fold Change | Regulation Status |
|---|---|---|---|---|---|---|
| **24h pathways** | | | **Cell Cycle** | | | |
| | CCNE2 | NM_057749 | cyclin E2 | 0.023 | 14,35 | down |
| | CDC45L | NM_003504 | cell division cycle 45 homolog | 0.037 | 22,75 | down |
| | | | **Integrated Breast Cancer Pathway** | | | |
| | IRS1 | NM_005544 | Insulin receptor substrate 1 | 0.012 | 0,75 | up |
| | | | **EGF-EGFR Signaling** | | | |
| | CAV2 | NM_001233 | caveolin 2 | 0.049 | 30,18 | up |
| | RIN1 | NM_004292 | Ras and Rab interactor 1 | 0.046 | 28,25 | up |
| **48h pathways** | | | **Integrated Breast Cancer Pathway** | | | |
| | IRS1 | NM_005544 | Insulin receptor substrate 1 | 0.003 | 1,86 | up |
| | HMGCR | NM_000859 | 3-hydroxy-3-methylglutaryl-CoA reductase | 0.006 | 0,04 | down |
| | BRCA2 | NM_000059 | Breast cancer 2, early onset | 0.001 | 1,02 | down |
| | RAD51 | NM_002875 | RAD51 homolog (S. cerevisiae) | 0.006 | 3,75 | down |
| | PAK1 | NM_001128620 | p21 protein (Cdc42/Rac)-activated kinase 1 | 0.008 | 5,16 | up |
| | | | **EGF-EGFR Signaling** | | | |
| | PAK1 | NM_001128620 | p21 protein (Cdc42/Rac)-activated kinase 1 | 0.008 | 5,16 | up |
| | E2F1 | NM_005225 | E2F transcription factor 1 | 0.003 | 1,83 | down |
| | STAT5A | NM_003152 | Signal transducer and activator of transcription 5A | 0.006 | 3,72 | up |
| | PLCE1 | NM_016341 | Phospholipase C, epsilon 1 | 0.004 | 2,47 | down |
| | FOS | NM_005252 | FBJ murine osteosarcoma viral oncogene homolog | 0.007 | 4,41 | down |
| | FOSB | NM_006732 | FBJ murine osteosarcoma viral oncogene homolog B | 0.007 | 4,83 | down |

Propolis at whole genome expression level on SKBR3

In addition to bioinformatic analysis performed in GeneSpring Software version7 13.0, gene enrichment analysis was achieved in String Program in order to investigate the gene interactions that were statistically significant. According to microarray data, it has been found that 210 gene expressions were statistically significantly changed after treatment of 50 µg/mL Anatolian propolis in SK-BR-3 cell line at 24th hour exposure ($p<0.05$). Among those 87 genes that were found down-regulated, 80 down-regulated genes have been detected in string analysis. Based on string analysis, down-regulated genes were located in beta tubulin superfamily, propanoate and pyruvate metabolism (Table 3). Also, it has been examined that 123 genes were detected up-regulated ($p<0.05$) and among those 120 up-regulated genes were recognized in string analysis. Up-regulated genes were found to be related with diverse pathway including response to stress, cellular response to chemical stimulus, immune system process, lipid metabolic process, nucleotide metabolic process, etc. (Supplement Table 3).

**Table 3.** 24th hour down- and up- regulated pathways (Enrichment KEGG) Interacting proteins with highest confidence were selected (0.900))

| #term ID | Term Description | Observed Gene Count | Background Gene Count | Strength | False Discovery Rate | Matching Proteins in Network |
|---|---|---|---|---|---|---|
| | | | ***Down regulated Pathways*** | | | |
| HSA00640 | Propanoate metabolism | 4 | 32 | 1.53 | 0.00077 | DLD, ACSS1, ACAT2, ECHDC1 |
| HSA00620 | Pyruvate metabolism | 3 | 139 | 1.32 | 0.0211 | DLD, ACSS1, ACAT2, |
| HSA05130 | Pathogenic E.Coli infection | 3 | 53 | 1.19 | 0.0327 | TUBA1, TUBB, TUBB2A |
| | | | ***Up regulated pathways*** | | | |
| HSA04913 | Ovarian steroidogenesis | 5 | 49 | 1.29 | 0.0014 | ALOX5, CYP1A1, AKR1C3, ADCY7, CYP1B1 |
| HSA05145 | Toxoplasmosis | 5 | 109 | 0.94 | 0.0252 | LAMC1, LY96, CD40, ALOX5, HSPA1A |

String analysis for the microarray data of 48th hour showed 343 down-regulated genes and 255 up-regulated genes. In string analysis, down-regulated genes in the 48th hour were found in pathways of Gene Ontology Biological process including cell cycle, cell cycle regulation, cell division, nuclear division, chromosome segregation, organelle fission, chromosome organization, DNA replication, sister chromatid segregation, phase transition of mitotic cell cycle, cytokinesis, meiotic cell cycle, initiation of DNA replication, organization of cell division , chromatin organization, DNA conformation change, organelle organization, microtubule cytoskeleton organization, cellular component regulation, control of protein serine/threonine kinase activity, DNA metabolic process, DNA repair, cellular response to DNA damage stimulus, cellular response to stress, signal transduction in response to DNA damage, cell cycle arrest due to signal transduction by p53 class mediator, cellular reaction to oxidative stress, mismatch repair, etc (Full pathways are given in Supplement Table 4). In contrast, after 48th hours of exposure to Anatolian propolis, up-regulated genes were found in different pathways such as immune system process, immune effector process, immune response, cytokine-mediated signaling pathway, response to interferon-gamma, leukocyte activation, oxidation-reduction process, response to organic substance, cellular response to chemical stimulus, antigen processing and presentation of exogenous peptide antigen via MHC class I, cellular reaction to xenobiotic stimulus, myeloid leukocyte activation, cell migration

regulation, organization of cell-cell adhesion, positive modulation of sprouting angiogenesis, etc (Full pathways are given in Supplement Table 5).

*3.3 Real time PCR Validation*

CCNB1, CCNB2, CDKN3, CDK1, BIRC5, E2F1, CDC25C and BIRC3 genes were selected for validation of the microarray data. CCNB1, CCNB2, CDKN3, CDK1, BIRC5, E2F1, CDC25C genes were found down-regulated in microarray data at the 48th hour of exposure to Anatolian propolis according to real time PCR findings and these selected genes were found down-regulated in the microarray analysis as well. BIRC3 gene was found up-regulated in both real time PCR and microarray analyses (Figure 2). These findings are validating our microarray data.

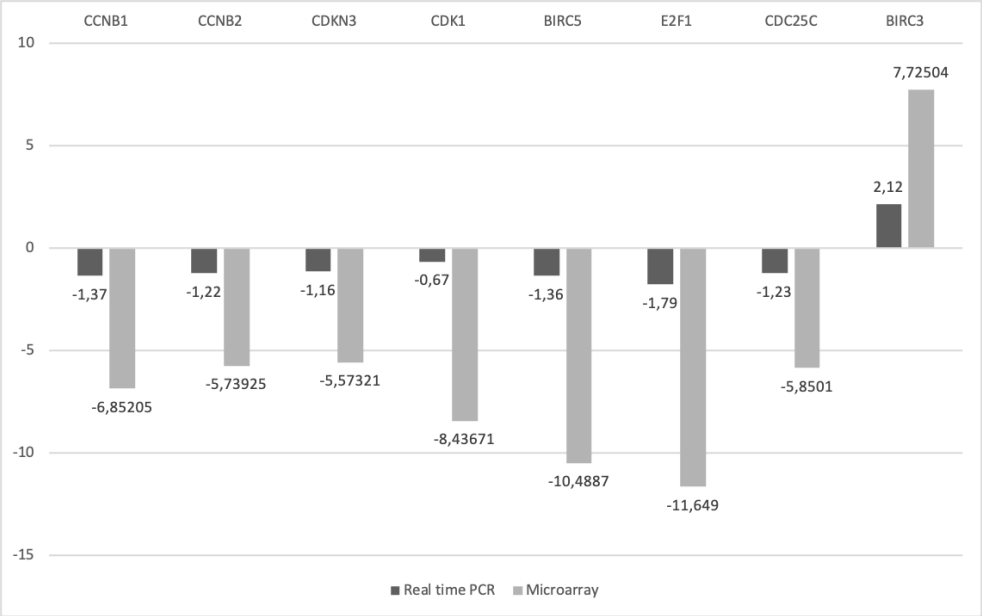


**Figure 2.** Gene expression comparison of Anatolian propolis at 48h on SK-BR-3 cells

Generally 15-20% of breast cancer cases are of the HER-2-positive breast cancer subtype, which is associated with high frequency of recurrence, poor prognosis and short survival [7, 18]. Overexpression of the HER-2 protein is observed in this subtype and mostly identified by immunohistochemistry, evaluating the copy number. HER-2 receptor is immensely expressed in different types of tissues on epithelial cells' membranes especially in the skin, breast and placenta, along with gastrointestinal, respiratory, reproductive and urinary track [19]. The pathogenesis of diverse solid tumors located in ovary, colon, lung, stomach and breast, is dramatically affected by the amplification or overexpression of HER-2 oncoprotein [20-23]. Trastuzumab (Herceptin), a monoclonal antibody that targets the extracellular area of the HER-2 receptors, is used in the therapy of HER-2-positive breast cancer patients [24]. During HER-2-targeted therapy, approximately 60-80 % of the patients develop resistance within a year, causing a huge clinical problem in treatment [25]. The important step for developing novel anti-HER-2 strategies is the identification and understanding of the trastuzumab resistance mechanisms [10,26]. Overexpression of HER-2 oncoprotein results with an intensive stimulation of downstream signaling pathways including phosphatidylinositol 3-kinase (PI3K), mammalian target of rapamycin (mTOR) and Ras/Raf/MEK/ERK pathways, which have functions in cellular metabolism, cell proliferation, migration and angiogenesis [17, 27-29].

Novel achievements and advancements in microarray technology have given an opportunity of investigating the gene profile of breast cancer subtypes with the intention of characterizing the patients, whom can be treated with adjuvant chemotherapy and radiotherapy; and eventually find biomarkers associated with prognosis [30, 31]. HER-2-positive breast cancers are resistant to current treatment and have highly heterogeneous subtypes, which are related to particular gene expressions or gene mutations

[32]. Based on the genomic identification of 64 HER-2-positive breast samples, HER-2-positive breast cancers are separated into four subgroups (A, B, C, D) regarding genomic markers like somatic mutations and copy-number changes or structural variations [32, 33]. Additionally, 20 important genes are identified due to their expression status. Among those 20, 13 genes are upregulated in HER-2-positive breast cancer, such as TP53, Dickkopf-1 (DKK1), MKi67, HER1/EGFR, matrix metalloproteinase 15 (MMP15), baculoviral IAP repeat-containing 5 (BIRC5), cyclin E1 (CCNE1), CCND1, prolyl endopeptidase (PREP), kinesin-like protein (KIF18A), origin recognition complex subunit 6 homolog-like (ORC6L), ATPase H+ transporting V0 subunit a4 (ATP6V0A4), and reticulon 4 interacting protein 1 (RTN4IP1). These upregulated genes are mostly associated with poor survival due to their functions especially in endorsing cell proliferation, progression, metastasis and aggressive nature [33]. On the other hand, genes such as phosphaditidylinositol-4,5-biphospate 3-kinase catalytic subunit alpha (PIK3CA), phosphatidylinositol 3-kinase regulatory subunit (PIK3R1), phosphatase and tensin homolog (PTEN), inositol polyphosphate 4-phosphatase type II (INPP4B), TP63 and those related with poor prognosis and survival were downregulated (33). In our study, gene expressions of BIRC5, MKi67, DKK1 and KIF18A were down-regulated following treatment for 48 hours with fold changes of -10.48872, -20.819077, -9.752987, 11.82791, respectively ($p < 0.0001$). This shows that Anatolian propolis altered expressions of these genes the opposite way after treatment for 48 hours. Especially, BIRC5 is known for its overexpression in HER-2 positive and triple negative breast cancers [34]. In this regard, dramatical reverse of BIRC5 expression SK-BR-3 cell line might have led the cancer cells to become apoptotic. It is well-known that MKi67 is utilized as a prognostic biomarker indicating proliferative function of tumor cells in different cancer types [35]. MKi67 gene expression oppositely changed as down regulation after exposure to Anatolian propolis in SK-BR-3 cells, which may demonstrate the anti-proliferative effect of Anatolian propolis. HER-2/neu causes inordinate cell division, raising the number of tumor-initiating cells (TICs) with relationed to mammary stem cells, therefore, its uninterrupted expression is needed to sustain carcinogenesis [36]. Liu et al. suggested that 17 genes expressions were found enriched HER-2/neu mammary TICs (8 upregulated and 9 down regulated genes) and they showed AURKB and CCNB1 genes were down regulated [37]. Similarly in our study, we have found that Anatolian propolis significantly down regulated AURKB and CCNB1 genes, 18. 92 and 16.85-fold, respectively. AURKB has a key role in the process of cell division and assembles with BIRC5 (Survivin) and inner centromere protein [38]. The overexpression has been correlated with aneuploidy in various cancers types such as breast, ovarian and prostate [39].

**Table 4.** The comparison of significantly changed genes in our study with literature (After treatment of 50 µg/mL doses of Anatolian propolis for 48 hours)

| **Our Study** | | | **Comparison with Literature** | | |
|---|---|---|---|---|---|
| **Gene Name** | **P Value** | **Fold Change** | **P Value** | **Regulation Status at Literature** | **Reference** |
| BIRC5 | 0,002 | -10,49 | 0,0023 | Up regulated in HER-2-positive breast cancers | [33] |
| MKi67 | 0,001 | -20,82 | 0,0152 | Up regulated in HER-2-positive breast cancers | [33] |
| DKK1 | 0,013 | -9,75 | 0,0131 | Up regulated in HER-2-positive breast cancers | [33] |
| KIF18A | 0,019 | -11,83 | 0,0196 | Up regulated in HER-2-positive breast cancers | [33] |
| AURKB | 0,028 | -18,92 | 0,0277 | Up regulated in HER-2/neu mammary tumor-initiating cells (TICs) | [36, 37] |
| CCNB1 | 0,029 | -5,74 | 0,0293 | Up regulated in HER-2/neu mammary tumor-initiating cells (TICs) | [36, 37] |

In the light of our findings, Anatolian propolis inhibited cell division and caused SK-BR-3 cells to apoptosis by switching the expression of AURKB and BIRC5. Furthermore, it has been suggested that KIF18A overexpression in breast cancer can be used as a predictive biomarker of lymph node metastasis [40]. Also, it was proposed that KIF18A overexpression may have significant function in breast cancer development due to its association with metastasis, poor survival and tumor grade [41]. Interestingly, KIF18A gene expression was down regulated after treatment, which points out that Anatolian propolis suppressed metastasis in breast cancer by inhibiting the KIF18A gene expression (Table 4).

When we compare our data regarding caffeic acid with literature, we have found only one research that has been focusing on SK-BR-3 cell line and they detected that IC50 value of caffeic acid is 261(± 0,7) µg/mL at 48$^{th}$ hour exposure [42]. On contrary in our data, caffeic acid showed remarkable inhibition at 24$^{th}$ hour exposure (IC50 value 13,00) and IC50 value was calculated at 48$^{th}$ exposure as 80.57. In our findings we have showed that pinostrobin decreased the cell viability after the amount of 15 µg/mL at all time intervals, with IC50 values of 24.65 at the 24$^{th}$ hour, 22.69 at the 48$^{th}$ hour and 19.15 at the 72$^{nd}$ hour. There is only one research that has investigated the effect of pinostrobin on SK-BR-3 cells and they found the IC50 value of pinostrobin as 94.3 µM [43]. In light of literature mining, there is no research that investigates the cell viability/cytotoxicity effect of galangin and kaempferol on SK-BR-3 breast cancer cell line that represents ER/PR-, HER-2/neu+ human breast cancer.

We have showed that polyphenol-rich Anatolian propolis has blocked the important pathways of HER-2 positive breast cancer cells including cell cycle, DNA repair and apoptosis. Also, we have showed for the first time the cell viability/cytotoxicity effect of galangin and kaempferol on SK-BR-3 cells. Starting from this point of view, we suggest that polyphenol-rich propolis can be used in the therapy of HER-2 positive breast cancer regarding it being significantly less toxic than the current treatment methods. In addition, polyphenolics mixtures and combinations can be developed as integrative molecular approaches in the therapy of various cancers including aggressive breast cancers by aiming multiple molecular steps not only focusing on one point.

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## Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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