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Gene Expression Analysis Shows Anticancer Mechanism of *Acacia podalyriifolia* Ethanol Extract on MCF7 Cells

(Analisis Ekspresi Gen Menunjukkan Mekanisme Antikanser Ekstrak Etanol *Acacia podalyriifolia* pada Sel MCF7)

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ABSTRACT

Breast cancer, particularly luminal A subtype, remains a significant challenge due to its resistance to certain therapies. Therefore, discovering new anticancer agents is critical. MCF7 cancer cells are commonly used as a model for studying luminal A breast cancer and screening potential anticancer compounds. This study aimed to investigate the anticancer potential of *Acacia podalyriifolia* leaves ethanol extract on MCF7 cells by exploring its effects on the expression of genes involved in the p53 signaling pathway. Differentially Expressed Genes (DEGs) analysis was performed on GSE208731 and GSE233242 obtained from the Gene Expression Omnibus (GEO) database to identify genes consistently regulated between MCF7 cells and luminal A breast cancer from patients or common DEG. The analysis identified several DEG, including CDKN1A (p21), GADD45A, CCNE1, CDK2, and E2F1, which are associated with the p53 signaling pathway. In this study, MCF7 cells were treated with IC_{50} dose of extract (154.52 µg/mL) was performed using the MTT assay. Then, gene expression analysis was performed using quantitative PCR (qPCR) to evaluate the impact on p53-related genes/common DEG. The extract significantly downregulated CCNE1 and E2F1, which are downstream genes in the p53 signaling pathway responsible for regulating the cell cycle. This downregulation led to cell cycle arrest and inhibited cell proliferation, suggesting the potential of *Acacia podalyriifolia* as a candidate anticancer agent for luminal A breast cancer.

Keywords: *Acacia podalyriifolia*; cell cycle arrest; differentially expressed genes; luminal A; MCF7 cells

ABSTRAK

Kanser payudara, terutamanya subjenis luminal A, kekal sebagai cabaran besar disebabkan oleh rintangannya terhadap terapi tertentu. Oleh itu, penemuan agen antikanser yang baharu adalah amat penting. Sel kanser MCF7 lazimnya digunakan sebagai model untuk mengkaji kanser payudara luminal A dan menyaring sebatian antikanser yang berpotensi. Penyelidikan ini bertujuan untuk mengkaji potensi antikanser ekstrak etanol daun *Acacia podalyriifolia* terhadap sel MCF7 dengan meneliti kesannya ke atas ekspresi gen yang terlibat dalam laluan isyarat p53. Analisis Gen Terekspresi Berbeza (DEGs) dijalankan ke atas set data GSE208731 dan GSE233242 yang diperoleh daripada pangkalan data Gene Expression Omnibus (GEO) untuk mengenal pasti gen yang dikawal secara tekal antara sel MCF7 dan sel kanser payudara luminal A daripada pesakit. Analisis ini mengenal pasti beberapa DEG, termasuk CDKN1A (p21), GADD45A, CCNE1, CDK2 dan E2F1 yang berkaitan dengan laluan isyarat p53. Dalam kajian ini, sel MCF7 dirawat dengan ekstrak dos IC_{50} (154.52 µg/mL) yang diperoleh melalui ujian MTT. Seterusnya, analisis ekspresi gen dilakukan menggunakan PCR kuantitatif (qPCR) untuk menilai kesan ke atas gen berkaitan p53/DEG bersama. Ekstrak tersebut didapati menurunkan ekspresi CCNE1 dan E2F1 dengan ketara, iaitu gen hiliran dalam laluan isyarat p53 yang bertanggungjawab mengawal kitaran sel. Pengawalaturan rendah ekspresi ini membawa kepada penahanan kitaran sel dan perencatan percambahan sel, menunjukkan potensi *A. podalyriifolia* sebagai calon agen antikanser untuk kanser payudara luminal A.

Kata kunci: *Acacia podalyriifolia*; gen yang diekspresikan berbeza; luminal A; penahanan kitaran sel; sel MCF7

INTRODUCTION

The incidence of breast cancer is increasing globally, with cases projected to reach 364,000 by 2040 (Arzanova & Mayrovitz 2022). This incidence has risen by 57.8% over the past 30 years, with an annual increase rate of 0.5% (Shang & Xu 2022). The most common and widely accepted classification of breast cancer is from an immunohistochemical perspective, based on the expression of the following hormone receptors: estrogen (ER), progesterone (PR), and human epidermal growth factor (HER2). Consequently, four widely recognized breast cancer subtypes are: luminal A, luminal B, HER2-positive, and triple-negative (Orrantia-Borunda et al. 2022). Luminal A breast cancer (HR positive/HER2 negative, HR+/HER2-) is estrogen-dependent, and therefore typically responds well to treatments that block estrogen receptor function. These treatments are often quite effective; however, over time, tumors evolve and develop resistance through various mechanisms (Anderson 2021). MCF7 cells are a luminal A subtype that express estrogen receptors (ER) and progesterone receptors (PR) (Moon et al. 2020). MCF7 cells have been extensively used in the research of ER-positive breast cancer cells, including studies in the search for breast cancer anti-cancer compounds (Rahmawati et al. 2018).

Understanding the molecular mechanisms underlying breast cancer is crucial for diagnosis and treatment. High-throughput genomic innovations, such as microarray analysis and RNA sequencing, have aided in identifying significant genes correlated with breast cancer and studying their interactions (Alshabi et al. 2019). Differential gene expression (Differentially Expressed Genes, DEG) analysis can be used to identify genes that are upregulated or downregulated in breast cancer, providing insights into the disease's mechanisms (Schagerholm et al. 2022; Zhang et al. 2022). Additionally, identifying hub genes within protein-protein interaction networks, biological pathway analysis, and gene ontology can reveal potential targets for diagnostic biomarkers and treatments.

Gene expression regulation plays a key role in cell cycle arrest, which is crucial in the behavior and treatment response of breast cancer cells. This cell cycle arrest influences tumor heterogeneity and drug resistance, particularly in phases such as G0 and G1. Breast cancer cells can enter a dormant state (G0) or become arrested in G1, which has been linked to increased resistance to apoptosis and enhanced survival under stress conditions. This process is often accompanied by increased autophagy, which aids cell survival during DNA damage (Kluska et al. 2023). Activation of the p53 signaling pathway in response to cellular stress induces significant downstream gene regulation, which halts cell proliferation. p53 directly activates the transcription of the CDKN1A gene, which encodes p21, a cyclin-dependent kinase inhibitor. Increased expression of p21 inhibits cyclin-CDK complexes, preventing RB phosphorylation, thus maintaining its repressive effect on the E2F transcription factor, and consequently arresting cell cycle progression.

Breast cancer treatment is an ever-evolving area of research, including the exploration of the potential of certain plants to influence gene expression involved in this condition. One genus of interest in this context is Acacia. Notable Acacia plants in medicinal use include *A. kempeana, A. ligulata, A. tetragonophylla, A. mearnsii*, and *A. pycnantha*, which are rich in polyphenolic compounds. Observed biological activities include antioxidant, antimicrobial, anticancer, α-glucosidase inhibition, and anti-inflammatory properties (Ung & Asmara 2023). Extracts of *A. laeta, A. hamulosa, A. tortilis*, and *A. seyal* have shown cytotoxic properties against breast cancer cells MCF7 and MDA-MB-231, with *A. seyal* extract known to induce apoptosis in MDA-MB-231 cells through the downregulation of Bcl-2 and Bcl-xL protein expression (Zingue et al. 2018). Methanol extract of *A. saligna* leaves has shown cytotoxic activity against MCF7, with rutin, hyperoside, quercetin, miquelianin, and p-coumaric acid identified as compounds present in the extract (Elansary et al. 2020). Rutin is known to induce apoptosis in TNBC MDA-MB-231 cells through the upregulation of ASK1 and JNK (Suganya et al. 2022). Additionally, hyperoside and p-coumaric acid have been shown to decrease the expression of Bcl-2 (an apoptosis inhibitor) and increase the expression of Bax in MCF7 cells (Qiu et al. 2019; Saremi et al. 2022).

Similar to other species in the Acacia genus, *Acacia podalyriifolia* is also suspected to have anticancer activity. This plant is believed to originate from Australia but is also found growing in Tawangmangu, Indonesia (Haryanti & Widiyastuti 2017). An infusion (water extract) of *A. podalyriifolia* leaves has been shown to exhibit cytotoxic activity against MCF7 cells (Haryanti & Widiyastuti 2017). However, there have been no studies on the anticancer activity of *A. podalyriifolia* and its effects on the gene expression changes related to its anticancer mechanisms. This study aims to determine the cytotoxic effects of *A. podalyriifolia* leaves ethanol extract on MCF7 breast cancer cells and the associated gene expression changes.

MATERIALS AND METHODS

EXTRACTION OF *A. podalyriifolia* LEAVES

The plants were obtained from the area referenced in the study by Haryanti and Widiyastuti (2017). Plant verification was carried out using a botanical guide that includes detailed descriptions of morphology, habitat, and taxonomy, as found on the website: https://keyserver.lucidcentral.org/weeds/data/media/Html/acacia_podalyriifolia.htm. *A. podalyriifolia* leaves were dried and powdered. Extraction was carried out using an ultrasonic method with ethanol 96% as the solvent. The extraction process lasted for 1 h. The solvent solution mixed with the simplisia was filtered to obtain the filtrate. The filtrate was then concentrated using a rotary evaporator.

DIFFERENTIALLY EXPRESSED GENES (DEG) ANALYSIS

The GSE208731 data obtained from the Gene Expression Omnibus (GEO) (Barrett et al. 2012) database underwent DEG analysis using GEO2R. The GSE208731 data contains RNA-seq data from 2D and 3D cultures of MCF7 and MCF10A cells within the same experiment. The DEGs identified from the GSE208731 data were then compared with the DEGs from the GSE233242 analysis to find intersecting DEGs between the two datasets. The GSE233242 dataset contains RNA-seq data obtained from luminal A breast tumors and normal human breast tissue. The aim of identifying intersecting or common DEGs between the two datasets is to find genes with similar expression patterns, so that the gene expression changes observed at the *in vitro* level are expected to also occur at the tumor tissue level obtained from patients. The common DEGs identified were then validated using Principal Component Analysis (PCA) and machine learning methods with the Support Vector Machine (SVM) algorithm. Validation was performed to obtain values for sensitivity, specificity, and area under the curve (AUC) as measures of the ability of the identified DEGs to distinguish normal breast cells (MCF10A) from breast cancer cells (MCF7) based on their DEG pattern changes.

CELL VIABILITY ASSAY (MICROTETRAZOLIUM, MTT ASSAY)

MCF7 cells at a density of 5×10^3 cells/100 µL were placed in a 96-well plate with a medium containing 10% FBS solution, penicillin-streptomycin, and DMEM. The MCF7 cells were then incubated with 100 µL *A. podalyriifolia* leaves ethanol extract in serial concentration (500;250;125;62.50;31.25;15.63;7.81;3.91;0.00 µg/mL) for 24 h in a 5% CO_2 incubator at 37 °C. Incubation was also performed on MCF7 cells given serial concentrations of tamoxifen as a positive control at the same concentration variation. After incubation, 100 µL of 5 mg/mL MTT solution was added to each well. The MTT assay reaction was then stopped by adding 10% SDS stopper after 6 h. The incubation process was resumed in the dark overnight at room temperature. The results of the MTT assay reaction were a purple color, and its absorbance was measurable using an ELISA reader at a wavelength of 595 nm. All experiments were performed in triplicate, and the results were presented as a percentage of cell inhibition compared to the control.

ANALYSIS OF GENE EXPRESSION USING REAL-TIME PCR (RT-PCR)

Gene expression measurement was conducted by extracting total RNA from MCF7 cells treated with the IC_{50} dose of the extract. The determination of the IC_{50} dose was performed using the MTT assay. Tamoxifen was administered to MCF7 cells at an IC_{50} dose (25.79 µg/mL), as referenced in Arsianti, Nur Azizah and Erlina (2023). From 200 ng of extracted RNA, reverse transcription into cDNA was performed and quantified using RT-PCR. Genes with the highest Log2(fold change, FC) from the DEG validation results were selected for expression measurement.

Total RNA Extraction and Reverse Transcription

Total RNA from MCF7 cells was extracted using the FastPure® Cell/Tissue Total RNA Isolation Kit V2 (Vazyme). Subsequently, 200 ng of the extracted RNA was reverse-transcribed into cDNA using the HiScript III RT SuperMix (Vazyme) prior to qRT-PCR. Quantitative PCR was performed using 2×Taq pro Universal SYBR qPCR Master Mix (Vazyme) on an FMR3 Real-Time Quantitative Thermal Cycler (Vazyme Medical Technology, Nanjing, China). The ACTB gene was employed as a housekeeping (HK) gene to normalize the expression of the target genes. Each sample was analyzed in triplicate. The thermocycling program included annealing at 94 °C for 2 min, followed by 30 cycles of 30 s at 94 °C, 30 s at 56 °C, and 60 s at 72 °C. Melting curve data were then collected to verify PCR specificity and the absence of primer dimers.

Gene Expression Analysis

The expression values of the genes were expressed relative to the housekeeping gene using the Livak and Schmittgen method (Livak & Schmittgen 2001). Relative quantification (R) was calculated as R = 2^(-ΔΔCt), where ΔΔCt = ΔCt_sample - ΔCt_control = (Ct_target – Ct_HK)_sample - (Ct_target – Ct_HK)_control (Pfaffl, Horgan & Dempfle 2002). Ct represents the cycle threshold, indicating the number of cycles required for the fluorescence signal to surpass or cross the threshold level. A T-test was conducted to examine the mean differences in gene expression between treatment groups (plant extract and tamoxifen) and the control.

RESULTS

The results of DEG analysis on the GSE208731 and GSE233242 datasets can be seen in Figures 1 and 2. It can be observed that both MCF7 cells and luminal A tumor tissue can be sufficiently distinguished from normal cells or tissues based on their gene expression profiles or DEGs. The common DEGs or intersection results of DEGs from both datasets were then associated with genes involved in the luminal A breast cancer pathway obtained from the KEGG database (hsa05224) (Kanehisa et al. 2023). The luminal A breast cancer pathway can be viewed in Figure 3. Meanwhile, the common DEGs related to this pathway along with their expression values in log2 (fold change, FC) format can be seen in Table 1.

In Table 1, rows highlighted in gray indicate genes with non-significant expression changes (Padj > 0.05). However, genes such as BRCA1, BRCA2, and MYC, also highlighted in gray, are well-known to be strongly associated with breast cancer development and thus are not the primary focus of this study. Several unhighlighted genes show expression changes based on log2 (fold change) values, including genes involved in Cell Cycle (G1/S) progression. These genes—CCND1, CCNE1, E2F1, and CDK2—exhibit increased expression

(positive log2(fold change) values) in both MCF-7 and Luminal A cells (shared DEG), playing a critical role in G1/S phase transition and supporting cell proliferation. Meanwhile, within the p53 signaling pathway, genes such as CDKN1A and GADD45A demonstrate decreased expression (negative log2(fold change) values). Other genes related to the p53 signaling pathway, such as CCNE1, E2F1, and CDK2, as noted earlier, show increased expression. The p53 signaling pathway is also recognized for its role in promoting cell proliferation.

In Figure 3, it can be seen that luminal A breast cancer also consists of several common pathways such as the p53 signaling pathway, cell cycle, and estrogen/progesterone signaling pathway. Pathways like the p53 signaling pathway and cell cycle, as depicted in Figure 3, also play a role in other breast cancer types such as luminal B, HER2 positive, and Basal like/triple negative. Thus, influencing the expression of genes related to the p53 signaling pathway and cell cycle appears to have the potential to be utilized as a strategy in broader spectrum breast cancer treatment. Based on these reasons, validation with machine learning and evaluation of *A. podalyriifolia* leaves ethanol extract *in vitro* on MCF7 cancer cells will be focused on genes associated with the p53 signaling pathway and cell cycle. Therefore, based on the data in Table 1, the selected genes are CDKN1A (p21), GADD45A, E2F1, CCNE1, and CDK2. These genes were chosen because they are classified as Differentially Expressed Genes (DEGs) and had consistent gene expression regulation values or had the same log2FC numerical sign in MCF7 cells and luminal A cancer tissue.

The results of PCA analysis and validation (accuracy, sensitivity, specificity, and AUC values) using the SVM prediction model can be seen in Figure 4 and Table 2. Based on Table 2, it can be observed that the accuracy of the SVM model in predicting the MCF7 and MCF10A groups was 100%. Thus, it can be concluded that the sensitivity, specificity, and AUC of the SVM model in predicting both groups based on the expression of CDKN1A (p21), GADD45A, E2F1, CCNE1, and CDK2 genes were all 1 or 100%. Therefore, the expression of these five genes can be used to evaluate the administration of *A. podalyriifolia* leaves ethanol extract on MCF7 cells.

The results of the viability assay of MCF7 cells treated with ethanol extract of *A. podalyriifolia* leaves can be seen in Figure 5. The IC_{50} value obtained from the MTT assay, which is 154.52 µg/mL, was used as the concentration dose of the ethanol extract of *A. podalyriifolia* leaves for measuring the mRNA expression of CDKN1A (p21), CycE (CCNE1), and E2F1, or the downstream genes of the p53 signaling pathway related to cell cycle arrest in MCF7 cells. The tamoxifen dose was referred to previous research, using an IC_{50} value of 25.79 µg/mL (Arsianti, Nur Azizah & Erlina 2023). The primers used were listed in Table 3, while the results of the relative mRNA expression quantification (R) can be seen in Figure 6.

In Figure 6(a) and 6(b), there was a significant difference in relative mRNA expression or Relative quantification (R) ($p<0.05$) for the genes CDKN1A (p21), CycE (CCNE1), and E2F1. The relative mRNA expression of CCNE1 and E2F1 with the administration of ethanol extract of *A. podalyriifolia* leaves showed downregulation compared to the control, while the mRNA expression of CDKN1A showed an upregulation. In the case of tamoxifen administration, only the mRNA expression of E2F1 showed downregulation.

DISCUSSION

Inhibition of the p53 signaling pathway is expected to affect downstream genes, including those related to cellular senescence or cell cycle arrest. In this context, p53 is responsible for controlling the cell cycle to maintain genetic integrity. When p53 levels are low, it induces cell cycle arrest, which halts the cell cycle for the process of genetic damage repair, leading to cellular senescence. In cancer cells, cellular senescence is a tumor-suppressive program marked by stable cell cycle arrest. Therefore, cell cycle arrest in cancer cells is a target mechanism for anti-tumor agents to prevent cell proliferation (Kumari & Jat 2021; Lessard et al. 2018).

Analysis of data sets GSE208731 and GSE233242 using GEO2R showed that cell cycle arrest-related genes such as CDKN1A (p21), GADD45A, E2F1, CCNE1, and CDK2 have consistent mRNA expression in luminal A breast cancer patients and MCF7 cells. Thus, the search for luminal A breast cancer candidates through cellular senescence or cell cycle arrest mechanisms can be evaluated *in vitro* in MCF7 cells based on the expression of these genes. In this study, CDKN1A (p21), CycE (CCNE1), and E2F1 showed significant downregulation of mRNA expression in MCF7 cells due to treatment with *A. podalyriifolia* leaves ethanol extract. In contrast, with tamoxifen treatment, only E2F1 and CDKN1A showed decreased mRNA expression. E2F1 and CCNE1 are genes whose expression can serve as markers in cancer cells or patients. E2F1 is a transcription factor that regulates cell cycle progression. E2F1 is highly expressed in most cancer cells and activates the transcription of cell cycle-related kinases (Fang et al. 2022). Moreover, patients with high CCNE1 expression have a poorer prognosis compared to those with low expression in ovarian cancer, breast cancer, gastric cancer, lung cancer, and liver cancer (Zheng et al. 2023). Significant down-regulation of CCNE1 and E2F1 expression with treatment of *A. podalyriifolia* leaves ethanol extract is relevant to the p53-mediated cellular senescence and cell cycle cascade, leading to cell cycle arrest. Therefore, the antiproliferative properties of the extract are likely through this mechanism. In contrast, tamoxifen appears to play a less significant role in influencing the p53-mediated cellular senescence and cell cycle pathways in MCF7.

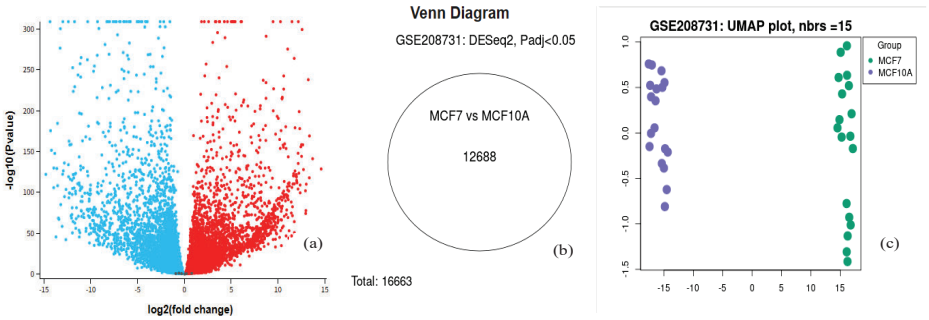


FIGURE 1. Results of DEG analysis on the GSE208731 dataset (a) The volcano plot depicts upregulated (red) and downregulated (blue) DEGs, (b) A total of 12688 DEGs were identified out of a total of 16683 mRNAs in MCF7 and MCF10A cells, and (c) Based on the DEGs generated, MCF7 cancer cell cultures can be distinguished from normal breast cell cultures MCF10A

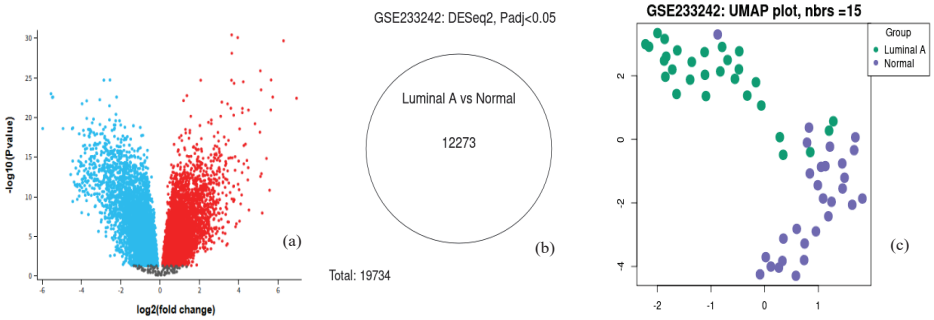


FIGURE 2. Results of DEG analysis on the GSE233242 dataset (a) The volcano plot depicts upregulated (red) and downregulated (blue) DEGs, (b) A total of 12273 DEGs were identified out of a total of 19734 mRNAs in luminal A breast cancer patient tumor tissue and normal breast tissue, and (c) Based on the DEGs generated, luminal A breast cancer patient tumor tissue and normal breast tissue can be sufficiently distinguished

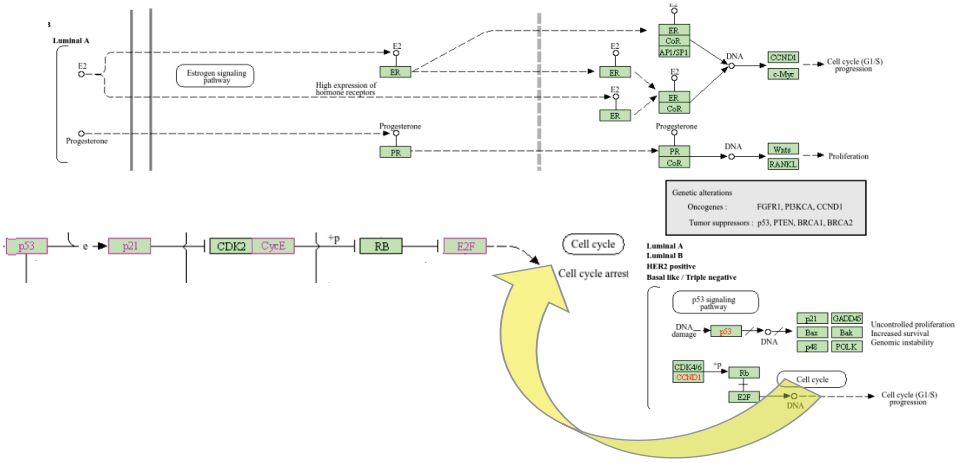


FIGURE 3. Luminal A breast cancer pathway (KEGG: hsa05224). Genes related to the cell cycle/cell cycle arrest pathway are also detailed in this figure

TABLE 1. Common DEGs related to the luminal a breast cancer pathway

Symbol	log2(fold change) MCF7	log2(fold change) LUMINAL A	Response	Pathway	Genetic Alteration
CCND1	1.219	1.285	Cell Cycle (G1/S) Progression	Estrogen Signaling Pathway	Oncogene
MYC	-1.955	-1.368			
WNT4/WNT1	not significant (P>0.05)	not significant (P>0.05)	Proliferation	Progesterone Signaling Pathway	Oncogene
RANKL (TNFSF11)	not significant (P>0.05)	not significant (P>0.05)			
FGFR1	1.868	-0.709			Oncogene
PI3KCA	not significant (P>0.05)	not significant (P>0.05)			
TP53 (p21)	not significant (P>0.05)	not significant (P>0.05)			Tumor Supressor
PTEN	-0.275	-0.295			
BRCA1	0.953	0.828			
BRCA2	1.648	1.719			
CDKN1A	-1.103	-1.232	Uncontrolled Proliferation, Genomic Instability, Increased Survival	p53 Signaling Pathway	Tumor Supressor
GADD45A	-2.836	-0.462			
BAX	0.487	0.374			
BAK	not significant (P>0.05)	not significant (P>0.05)			
DDB2 (p48)	not significant (P>0.05)	not significant (P>0.05)			
POLK	not significant (P>0.05)	not significant (P>0.05)			
E2F1	1.697	1.503	Cell Cycle (G1/S) Progression	p53 Signaling Pathway	Oncogene
RB1	-0.376	0.349			
CCNE1	0.426	0.896			
CDK2	1.579	0.312			

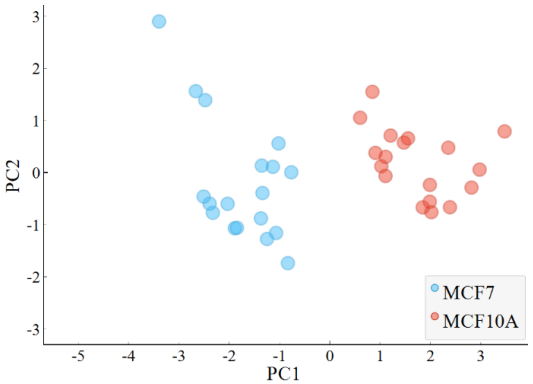
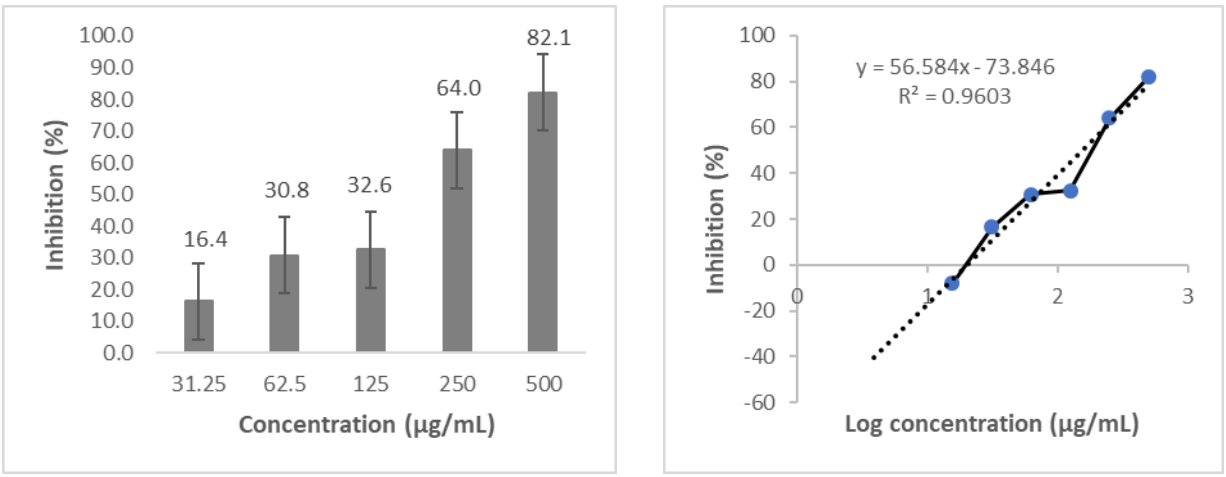


FIGURE 4. Scatter plot of Principal Component 1 (PC1) and PC2 values constructed by the expression of CDKN1A (p21), GADD45A, E2F1, CCNE1, and CDK2 genes. It can be observed that the combined expression of these five genes can distinguished between MCF7 and MCF10A cells

TABLE 2. The accuracy of the SVM model in predicting the MCF7 and MCF10A groups

		Predicted		
		MCF7	MCF10A	Total
Actual	MCF7	18	0	18
	MCF10A	0	18	18
	Total	18	18	36



(a) (b)

FIGURE 5. Viability assay of MCF7 cells treated with ethanol extract of *A. podalyriifolia* leaves at various concentrations. Based on (b), the IC_{50} value of the extract was determined to be 154.52 µg/mL

TABLE 3. Primers for downstream genes of the p53 signaling pathway related to cell cycle arrest

Assay set	Type	Sequence
CDKN1A	Forward Primer	GAGTGTAGGGTGTAGGGAGATT
	Reverse Primer	AGGAGGGAATTGGAGAGACTAC
CCNE1	Forward Primer	TCCCAAAGTGCTGGGATTAC
	Reverse Primer	CTACAAGCCTGAGCCACTATAC
E2F1	Forward Primer	GGTGAGAGCACTTCTGTCTTAAA
	Reverse Primer	CACCAAAGAGGCCTCGATAAA
ACTB (house keeping)	Forward Primer	TGACGTGGACATCCGCAAAG
	Reverse Primer	CTGGAAGGTGGACAGCGAGG

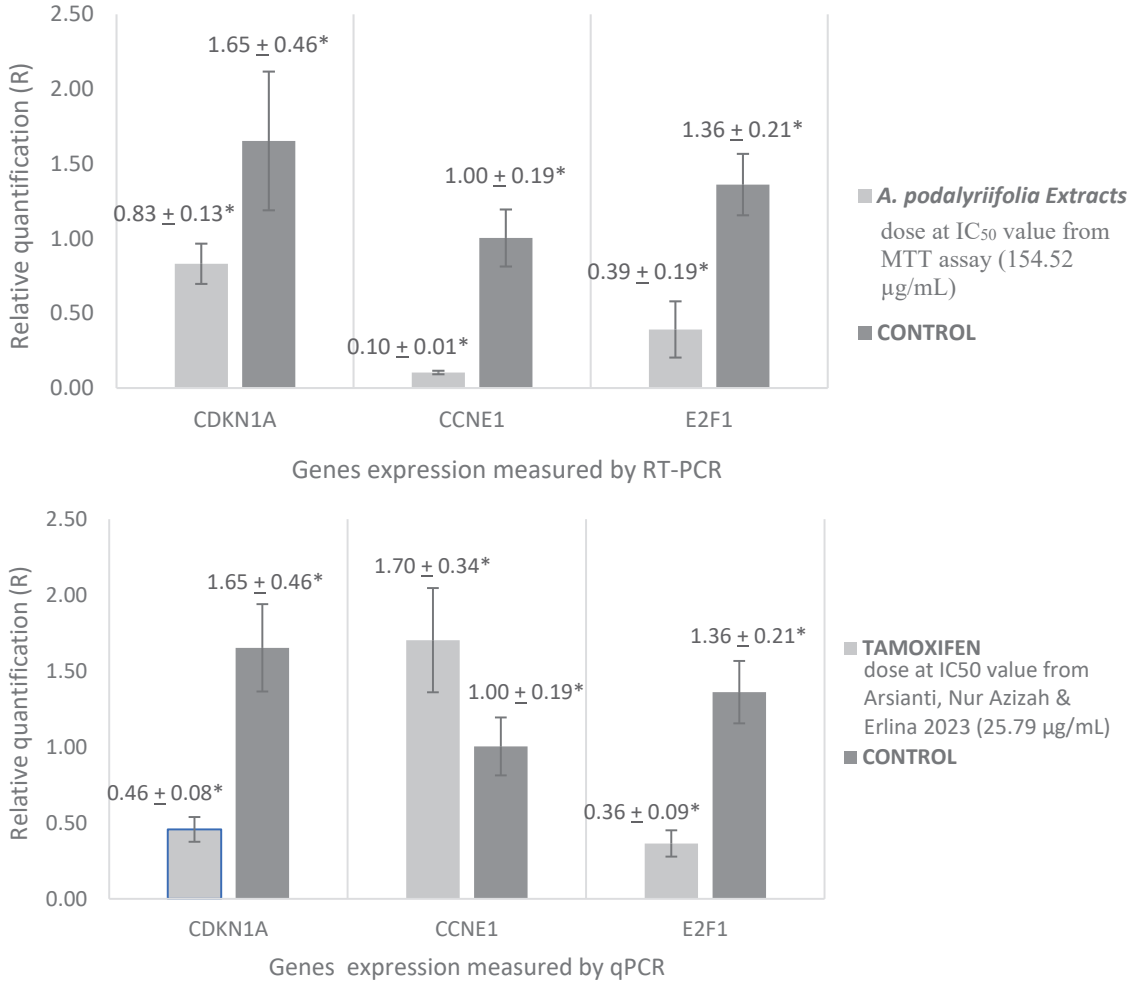


FIGURE 6. Relative mRNA expression or Relative quantification (R) between (a) *A. podalyriifolia* leaves extract and (b) tamoxifen compared to control, expressed as $2^{(-\Delta\Delta Ct)}$. The asterisk (*) indicates a significant difference ($p<0.05$) between the treatment of MCF7 cells and the control (untreated)

CDKN1A (p21) is a major inhibitor of p53-dependent apoptosis (Gartel & Tyner 2002). Overexpression of p21 was known to protect cells from apoptosis induced by doxorubicin (AbuHammad & Zihlif 2013). This gene is a negative regulator of p53, where knockdown of CDKN1A will upregulate p53, for instance, by doxorubicin induction (Broude et al. 2007). A 9.4-fold increase in CDKN1A expression was also known to occur in MCF7/D320 cells or doxorubicin-resistant MCF7 cells (Bunz et al. 1999). In this study, both tamoxifen and *A. podalyriifolia* leaves ethanol extract reduced CDKN1A mRNA expression. Therefore, the administration of the extract and tamoxifen may also play a role in inhibiting the protective effect of CDKN1A (p21) against apoptosis in MCF7 cells.

The results of this study indicate that the expression of genes involved in the cell cycle, such as CDKN1A, CCNE1, and E2F1, could serve as potential targets for new therapies. These three genes play a crucial role in regulating cell cycle arrest, a process in which cells cease division in response to damage or specific signals. It is well-known that current treatments for luminal A breast cancer, such as hormone therapy or chemotherapy, still face several limitations. First, hormone therapy often leads to resistance after prolonged use. Patients who initially respond to treatment may experience recurrence as cancer cells evolve and become resistant to the effects of hormone therapy. Additionally, chemotherapy has significant side effects and lacks selectivity, as it also affects healthy cells. Developing drugs that specifically target these genes may help create more effective and selective therapies for luminal A breast cancer, address treatment resistance, and reduce the side effects experienced by patients.

CONCLUSIONS

Analysis of Differentially Expressed Genes (DEGs) showed that the mRNA expression of CDKN1A (p21), GADD45A, E2F1, CCNE1, and CDK2 exhibited consistent regulation in luminal A breast cancer patients and MCF7 cells. Therefore, the expression of these genes can be used to evaluate luminal A breast cancer candidates *in vitro* in MCF7 cells. Treatment with *Acacia podalyriifolia* leaves ethanol extract on MCF7 cells resulted in the downregulation of CCNE1 and E2F1 mRNA, inducing cell cycle arrest and potentially playing a role in preventing cell proliferation.

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REFERENCES

Kluska et al. 2023

AbuHammad, S. & Zihlif, M. 2013. Gene expression alterations in doxorubicin resistant MCF7 breast cancer cell line. *Genomics* 101(4): 213-220. https://doi.org/10.1016/j.ygeno.2012.11.009

Alshabi, A.M., Vastrad, B., Shaikh, I.A. & Vastrad, C. 2019. Exploring the molecular mechanism of the drug-treated breast cancer based on gene expression microarray. *Biomolecules* 9(7): 282. https://doi.org/10.3390/biom9070282

Anderson, D.H. 2021. Luminal A breast cancer resistance mechanisms and emerging treatments. In *Biological Mechanisms and the Advancing Approaches to Overcoming Cancer Drug Resistance* Elsevier. pp. 1-22. https://doi.org/10.1016/B978-0-12-821310-0.00010-3

Arsianti, A., Nur Azizah, N. & Erlina, L. 2023. Molecular docking, ADMET profiling of gallic acid and its derivatives (N-alkyl gallamide) as apoptosis agent of breast cancer MCF-7 cells. *F1000Research* 11: 1453-1479. https://doi.org/10.12688/f1000research.127347.2

Arzanova, E. & Mayrovitz, H.N. 2022. The epidemiology of breast cancer. In *Breast Cancer.* Brisbane: Exon Publications. pp. 1-20. https://doi.org/10.36255/exon-publications-breast-cancer-

Barrett, T., Wilhite, S.E., Ledoux, P., Evangelista, C., Kim, I.F., Tomashevsky, M., Marshall, K.A., Phillippy, K.H., Sherman, P.M., Holko, M., Yefanov, A., Lee, H., Zhang, N., Robertson, C.L., Serova, N., Davis, S. & Soboleva, A. 2012. NCBI GEO: Archive for functional genomics data sets - update. *Nucleic Acids Research* 41(D1): D991-D995. https://doi.org/10.1093/nar/gks1193

Broude, E.V., Demidenko, Z.N., Vivo, C., Swift, M.E., Davis, B.M., Blagosklonny, M.V. & Roninson, I.B. 2007. p21 (CDKN1A) is a negative regulator of p53 stability. *Cell Cycle (Georgetown, Tex.)* 6(12): 1468-1471.

Bunz, F., Hwang, P.M., Torrance, C., Waldman, T., Zhang, Y., Dillehay, L., Williams, J., Lengauer, C., Kinzler, K.W. & Vogelstein, B. 1999. Disruption of p53 in human cancer cells alters the responses to therapeutic agents. *Journal of Clinical Investigation* 104(3): 263-269. https://doi.org/10.1172/JCI6863

Elansary, H.O., Szopa, A., Kubica, P., Ekiert, H., Al-Mana, F.A. & Al-Yafrsi, M.A. 2020. Antioxidant and biological activities of acacia saligna and lawsonia inermis natural populations. *Plants* 9(7): 908-925. https://doi.org/10.3390/plants9070908

Fang, Z., Lin, M., Chen, S., Liu, H., Zhu, M., Hu, Y., Han, S., Wang, Y., Sun, L., Zhu, F., Xu, C. & Gong, C. 2022. E2F1 promotes cell cycle progression by stabilizing spindle fiber in colorectal cancer cells. *Cellular & Molecular Biology Letters* 27(1): 90-111. https://doi.org/10.1186/s11658-022-00392-y

Gartel, A.L. & Tyner, A.L. 2002. The role of the cyclin-dependent kinase inhibitor p21 in apoptosis. *Molecular Cancer Therapeutics* 1(8): 639-649.

Haryanti, S. & Widiyastuti, Y. 2017. Aktivitas sitotoksik pada sel MCF-7 dari tumbuhan Indonesia untuk pengobatan tradisional kanker payudara. *Media Penelitian dan Pengembangan Kesehatan* 27(4): 247-254.

Kanehisa, M., Furumichi, M., Sato, Y., Kawashima, M. & Ishiguro-Watanabe, M. 2023. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Research* 51(D1): D587-D592. https://doi.org/10.1093/nar/gkac963

Kumari, R. & Jat, P. 2021. Mechanisms of cellular senescence: Cell cycle arrest and senescence associated secretory phenotype. *Frontiers in Cell and Developmental Biology* 9: 645593. https://doi.org/10.3389/fcell.2021.645593

Lessard, F., Igelmann, S., Trahan, C., Huot, G., Saint-Germain, E., Mignacca, L., Del Toro, N., Lopes-Paciencia, S., Le Calvé, B., Montero, M., Deschênes-Simard, X., Bury, M., Moiseeva, O., Rowell, M.C., Zorca, C.E., Zenklusen, D., Brakier-Gingras, L., Bourdeau, V., Oeffinger, M. & Ferbeyre, G. 2018. Senescence-associated ribosome biogenesis defects contributes to cell cycle arrest through the Rb pathway. *Nature Cell Biology* 20(7): 789-799. https://doi.org/10.1038/s41556-018-0127-y

Livak, K.J. & Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods* 25(4): 402-408. https://doi.org/10.1006/meth.2001.1262

Moon, H.R., Ospina-Muñoz, N., Noe-Kim, V., Yang, Y., Elzey, B.D., Konieczny, S.F. & Han, B. 2020. Subtype-specific characterization of breast cancer invasion using a microfluidic tumor platform. *PLoS ONE* 15(6): e0234012. https://doi.org/10.1371/journal.pone.0234012

Orrantia-Borunda, E., Anchondo-Nuñez, P., Acuña-Aguilar, L.E., Gómez-Valles, F.O. & Ramírez-Valdespino, C.A. 2022. Subtypes of breast cancer. In *Breast Cancer*. Brisbane: Exon Publications. pp. 31-42. https://doi.org/10.36255/exon-publications-breast-cancer-epidemiology

Pfaffl, M.W., Horgan, G.W. & Dempfle, L. 2002. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Research* 30(9): e36. https://doi.org/10.1093/nar/30.9.e36

Qiu, J., Zhang, T., Zhu, X., Yang, C., Wang, Y., Zhou, N., Ju, B., Zhou, T., Deng, G. & Qiu, C. 2019. Hyperoside induces breast cancer cells apoptosis via ROS-mediated NF-κB signaling pathway. *International Journal of Molecular Sciences* 21(1): 131-145. https://doi.org/10.3390/ijms21010131

Rahmawati, Y., Setyawati, Y., Widodo, I., Ghozali, A. & Purnomosari, D. 2018. Molecular subtypes of Indonesian breast carcinomas - Lack of association with patient age and tumor size. *Asian Pacific Journal of Cancer Prevention* 19(1): 161-166. https://doi.org/10.22034/APJCP.2018.19.1.161

Saremi, S., Kolahi, M., Tabandeh, M. & Hashemitabar, M. 2022. Induction of apoptosis and suppression of Ras gene expression in MCF human breast cancer cells. *Journal of Cancer Research and Therapeutics* 18(4): 1052-1060. https://doi.org/10.4103/jcrt.JCRT_624_20

Schagerholm, C., Robertson, S., Sifakis, E.G., Hases, L., Williams, C. & Hartman, J. 2022. 223P Gene expression profiles in endocrine-resistant breast cancer. *Annals of Oncology* 33: S640. https://doi.org/10.1016/j.annonc.2022.07.262

Shang, C. & Xu, D. 2022. Epidemiology of breast cancer. *Oncologie* 24(4): 649-663. https://doi.org/10.32604/oncologie.2022.027640

Suganya, K., Poornima, A., Sumathi, S., Chigurupati, S., Alyamani, N.M., Ghazi Felemban, S., Bhatia, S., Al-Harrasi, A. & Sayed Moawad, A. 2022. Rutin induces endoplasmic reticulum stress-associated apoptosis in human triple-negative breast carcinoma MDA-MB-231 cells - *In vitro* and *in silico* docking studies. *Arabian Journal of Chemistry* 15(9): 104021-104035. https://doi.org/10.1016/j.arabjc.2022.104021

Ung, A.T. & Asmara, A.P. 2023. Bioactive phytochemicals of *Acacia saligna. Molecules* 28(11): 4396-4425. https://doi.org/10.3390/molecules28114396

Zhang, W., Liu, L., Zhao, S., Chen, L., Wei, Y., Chen, W. & Ge, F. 2022. Research progress on RNA-binding proteins in breast cancer (Review). *Oncology Letters* 23(4): 121-135. https://doi.org/10.3892/ol.2022.13241

Zheng, X., Chen, L., Liu, W., Zhao, S., Yan, Y., Zhao, J., Tian, W. & Wang, Y. 2023. CCNE1 is a predictive and immunotherapeutic indicator in various cancers including UCEC: A pan-cancer analysis. *Hereditas* 160(1): 13-30. https://doi.org/10.1186/s41065-023-00273-0

Zingue, S., Michel, T., Cisilotto, J., Tueche, A.B., Ndinteh, D.T., Mello, L.J., Njamen, D. & Creczynski-Pasa, T.B. 2018. The hydro-ethanolic extract of *Acacia seyal* (Mimosaceae) stem barks induced death in an ER-negative breast cancer cell line by the intrinsic pathway of apoptosis and inhibited cell migration. *Journal of Ethnopharmacology* 223: 41-50. https://doi.org/10.1016/j.jep.2018.05.021

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