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Effects of Bauhinia purpurea leaf extracts on cytotoxicity and CCND1 gene expression in MCF-7 cells

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ABSTRACT: Background: Breast cancer ranks as a major cause of cancer-related mortality among women worldwide, with *CCND1* (Cyclin D1) gene overexpression contributing significantly to disease progression. *Bauhinia purpurea*, a medicinal plant rich in bioactive compounds, has shown antioxidant, hepatoprotective, hypoglycemic, and antiproliferative properties, highlighting its potential as an anticancer agent. Methods: This study assessed the cytotoxic potential and modulation of *CCND1* gene expression by *B. purpurea* leaf extracts obtained using 96% ethanol, ethyl acetate, and n-hexane, tested in vitro on MCF-7 breast cancer cells. Cytotoxicity was measured through the MTT assay following 48 h of treatment, while CCND1 expression levels were analyzed via qRT-PCR with GAPDH as the internal control. Results: Phytochemical analysis identified flavonoids, tannins, polyphenols, steroids/triterpenoids, and alkaloids within the extracts. After 48 h, the MTT assay indicated that the ethyl acetate fraction exhibited moderate cytotoxicity (IC50 = 88.5 µg/mL), the n-hexane fraction showed weak cytotoxicity (IC_{50} = 271.8 µg/mL), and the ethanol fraction displayed no cytotoxic effect (IC_{50} = 979.99 µg/mL). Gene expression analysis showed that all extracts significantly reduced CCND1 levels at IC_{25}, IC_{50}, and IC_{75}, with effects comparable to doxorubicin ($p < 0.05$). Conclusion: *Bauhinia purpurea* leaf extracts showed solvent-dependent cytotoxicity and downregulated *CCND1* gene expression comparable to doxorubicin, highlighting its potential as a phytopharmaceutical for breast cancer therapy via cell cycle inhibition.

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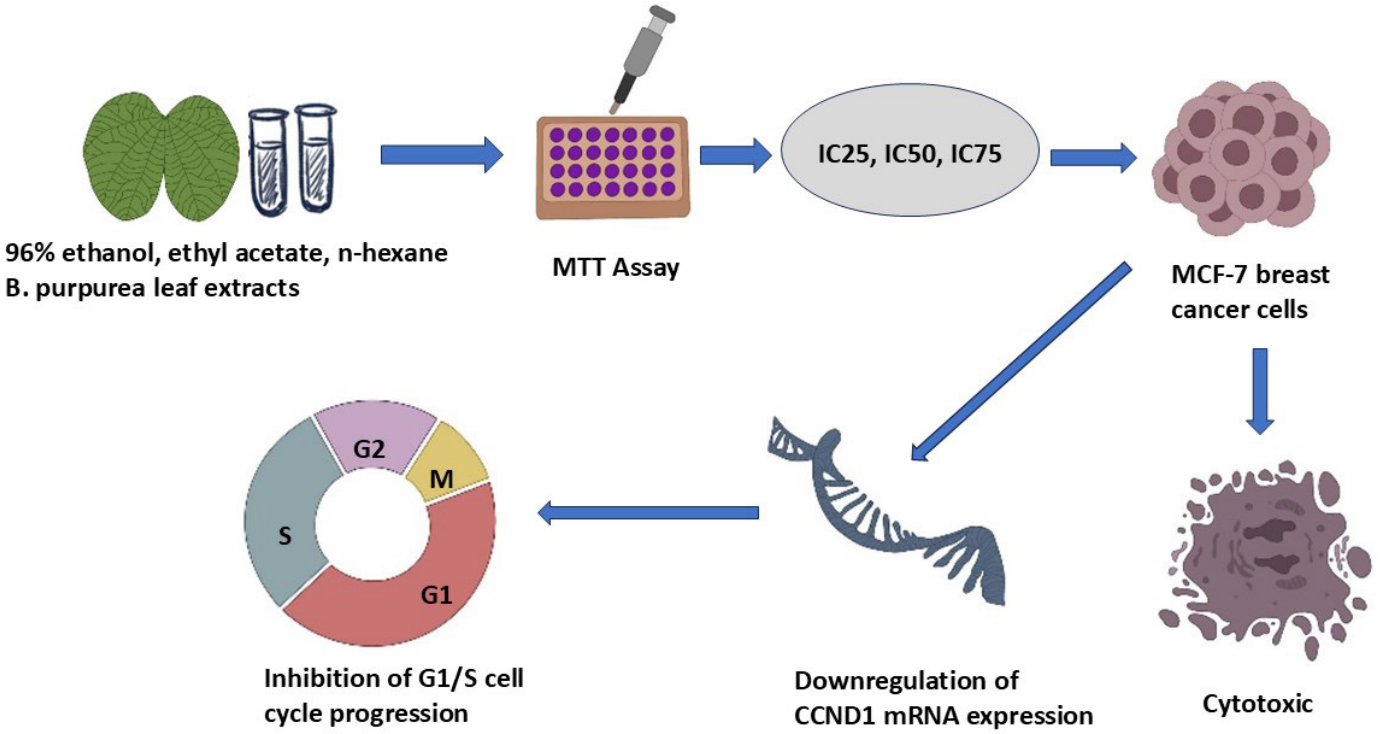
GRAPHICAL ABSTRACT



1. INTRODUCTION

Breast cancer continues to represent a major public health concern globally, ranking among the leading causes of cancer mortality in women and accounting for approximately 6.9% of all cancer-related deaths in 2020 (Arzanova & Mayrovitz, 2022). The therapeutic limitations and adverse side effects associated with conventional chemotherapeutic regimens have driven the search for alternative strategies based on bioactive compounds derived from natural sources.

Bauhinia purpurea, a species of the *Bauhinia* genus, has long been recognized in traditional medicinal systems for its effectiveness in addressing a broad range of ailments. Pharmacological evidence supports its wide-ranging biological activities, including antioxidant, hepatoprotective, hypoglycemic, and antiproliferative effects. Phytochemical profiling has demonstrated that this plant contains diverse secondary metabolites, such as glycosides, saponins, phenolics, tannins, flavonoids, flavonol glycosides, fatty acids, tocopherols, carbohydrates, alkaloids, sterols, steroids, flavanones, lutein, and beta-sitosterol (Arora et al., 2020).

The *CCND1* gene, situated at the 11q13 locus of chromosome 11, encodes the Cyclin D1 protein with a molecular mass of approximately 36 kDa. Functioning as a proto-oncogene, CCND1 plays a key role in driving cell cycle progression from G1 to S phase. Cyclin D1 interacts with holoenzyme kinases as a regulatory subunit, leading to the phosphorylation and subsequent inactivation of the retinoblastoma (Rb) protein, thereby enabling S phase initiation and stimulating cellular proliferation. Aberrant upregulation or gene amplification of Cyclin D1 has been strongly implicated in the molecular pathogenesis of numerous malignancies, notably breast cancer (Siraj et al., 2021).

MCF-7 breast cancer cells fall under the luminal A molecular subtype, defined by the expression of estrogen and progesterone receptors without overexpression of HER2. Their consistent molecular phenotype and responsiveness to hormonal cues make them an established in vitro system for exploring fundamental processes in breast cancer biology, including proliferative capacity, apoptotic pathways, and transcriptional regulation. Employing such cellular models is critical in preclinical research for assessing novel therapeutic candidates and exploring the molecular mechanisms underlying their pharmacological actions (Witt & Tollefsbol, 2023).

The polarity of solvents plays a significant role in determining the qualitative and quantitative extraction profile of bioactive compounds from botanical materials. Solvents with polar, semi-polar, or non-polar properties selectively dissolve distinct classes of phytoconstituents, which in turn shape the chemical composition and biological performance of the

resulting extracts. Therefore, choosing the optimal solvent is crucial to optimizing the therapeutic efficacy of plant-based extracts (Lee et al., 2024).

This research assessed the cytotoxic potential of *Bauhinia purpurea* leaf extracts obtained with solvents of different polarities (96% ethanol, ethyl acetate, and n-hexane) and examined their influence on *CCND1* gene expression in MCF-7 breast cancer cells under in vitro conditions.

2. MATERIALS AND METHODS

2.1. Research design

This in vitro study employed a post-test-only control group design to assess the cytotoxic effects and modulation of *CCND1* gene expression by *Bauhinia purpurea* leaf extracts prepared with various solvents on MCF-7 breast cancer cells. Thirteen experimental groups were established, including a negative control, doxorubicin as a positive control, and three extract fractions tested at IC_{25}, IC_{50}, and IC_{75} concentrations, enabling comparative analysis of gene expression across treatments. Gene expression quantification by qRT-PCR was performed using three biological replicates and three technical replicates per sample, with GAPDH as the reference gene, to ensure accuracy and consistency of the data.

2.2. Sample collection

Bauhinia purpurea leaves were collected from Sidoarjo Regency, East Java, Indonesia. MCF-7 breast cancer cells were provided by the Cell Culture Unit Biomedical Laboratory, Faculty of Medicine, Padjadjaran University, Indonesia. Plant identification was initially conducted through morphological analysis at the Biosystematics and Molecular Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Indonesia (Ref. No. 513/LBM/IT/III/2025), and subsequently confirmed through molecular analysis using rbcL (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) gene sequencing performed at PT Genetika Science Indonesia (Sample ID: G-3727-1).

2.3. Extraction process

The dried leaves of *Bauhinia purpurea* were ground into a fine powder using a mechanical grinder and subsequently sieved through a mesh-40 filter to ensure uniform particle size. Maceration was performed separately for each solvent—96% ethanol, ethyl acetate, and n-hexane—using a solvent-to-sample ratio of 3:1 (v/w). The mixtures were left to macerate for 72 hours at room temperature with occasional stirring to enhance extraction efficiency. After maceration, the extracts were filtered through Whatman No. 1 filter paper to remove solid residues. The filtrates were concentrated using a rotary evaporator to remove solvents, followed by further concentration in a water bath until semi-solid extracts were obtained.

2.4. Phytochemical analysis

Phytochemical characterization of *Bauhinia purpurea* leaf extracts prepared with ethanol, ethyl acetate, and n-hexane was undertaken through standard qualitative assays targeting secondary metabolites. To identify specific compound groups, Dragendorff's and Mayer's reagents were used for alkaloids, ferric chloride ($FeCl_3$) solution for polyphenols, 1% gelatin and Stiasny tests for tannins, the amyl alcohol assay for flavonoids, potassium hydroxide (KOH) for quinones, the foam test with hydrochloric acid (HCl) for saponins, vanillin–sulfuric acid for monoterpenoids and sesquiterpenoids, and the Liebermann–Burchard reagent for steroids and triterpenoids. Observations of color alterations or precipitate formation were taken as evidence of positive results.

2.5. Evaluation of cytotoxicity using MTT assay

Complete RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin was prepared for cell culture. *Bauhinia purpurea* leaf extracts were first dissolved in 100% dimethyl sulfoxide (DMSO) to create a 100,000 µg/mL stock solution, which was then serially diluted with culture medium to achieve working concentrations ranging from 100 to 2000 µg/mL. MCF-7 cells at a minimum of 80% confluence were harvested by trypsin-EDTA treatment, centrifuged, and resuspended in fresh medium. Cell viability and counts were determined using the trypan blue exclusion method. Subsequently, 5,000 viable cells were seeded per well in 96-well plates containing 200 µL of medium and incubated for 24 hours at 37°C with 5% CO_2 in a humidified incubator. After incubation, the medium was replaced with 200 µL of the respective extract dilutions in triplicate wells, followed by an additional 48-hour incubation under the same conditions. Following treatment, wells were washed with phosphate-buffered saline (PBS), and 100 µL of MTT reagent diluted in medium was added to each well. Plates were then incubated for 2 to 4 hours to allow for the formation of formazan crystals. The MTT solution was

removed, and DMSO was added to dissolve the crystals. Absorbance was measured at 550 nm using an ELISA microplate reader to assess cell viability.

2.6. Treatment for gene expression analysis

The MCF-7 cell line was grown in a complete RPMI-1640 medium containing 10% fetal bovine serum (FBS) and supplemented with 1% penicillin–streptomycin. When cell growth reached approximately 80% confluence, the culture was rinsed once with PBS, detached using 1 mL trypsin–EDTA for 5 minutes, and transferred into a centrifuge tube containing fresh complete medium. The cells were collected by centrifugation at 1500 rpm for 5 minutes, after which the pellet was resuspended in fresh medium. Cell number and viability were assessed using trypan blue exclusion with a haemocytometer. Subsequently, cells were seeded into 12-well plates at a density of 25,000–50,000 cells per well (1 mL/well) in triplicate for each group. After 24 hours of incubation at 37 °C in 5% CO_2, allowing them to reach ≥80% confluence, cells were exposed to the respective treatments or controls for 48 hours. Finally, cells were harvested by scraping, transferred into labeled centrifuge tubes, and immediately processed for RNA isolation and qRT-PCR at the Molecular Genetics Laboratory.

2.7. RNA isolation and qRT-PCR

RNA extraction from cultured MCF-7 cells was conducted using the Quick-RNA™ MiniPrep Plus Kit (Zymo Research, USA) following the manufacturer's protocol. Complementary DNA (cDNA) was synthesized and subjected to quantitative real-time reverse transcription PCR (qRT-PCR) with the SensiFAST™ SYBR® No-ROX One-Step Kit (Bioline, UK). The relative expression of the Cyclin D1 (*CCND1*) gene was determined using the comparative 2^−ΔΔCT method (Livak & Schmittgen), employing GAPDH as the housekeeping control. Each experiment was performed in triplicate to confirm data reliability. The primers used for CCND1 amplification were: forward 5′-TCTACACCGACAACTCCATCCG-3′ and reverse 5′-TCTGGCATTTTGGAGAGGAAGTG-3′.

2.8. Data analysis

All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to evaluate variations in relative gene expression among the groups, with Tukey's post hoc test applied for multiple group comparisons. A probability value (p) of less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Qualitative assessment of phytoconstituents in *Bauhinia purpurea* leaf extracts

Qualitative phytochemical analysis (Table 1) revealed that the 96% ethanol extract of *Bauhinia purpurea* leaves contained polyphenols, tannins, flavonoids, and steroids/triterpenoids. The ethyl acetate extract showed the presence of flavonoids and steroids/triterpenoids, while the n-hexane extract was found to contain alkaloids, polyphenols, and steroids/triterpenoids. These differences in phytoconstituents can be attributed to the polarity of the solvents, with ethanol being polar, ethyl acetate semi-polar, and n-hexane non-polar (Yani, Nastiti, & Noval, 2023).

Flavonoids are well known for their anticancer properties, acting by suppressing cell proliferation and promoting apoptosis through the regulation of diverse signaling pathways such as PI3K/Akt, MAPK, and NF-κB (Kopustinskiene et al., 2020; Sharma et al., 2022). Among tannins, tannic acid, a hydrolyzable polyphenolic compound, has demonstrated potent anticancer activity by downregulating VEGF and EGFR, inducing apoptosis via mitochondrial pathways, and modulating various signaling cascades such as EGFR/STAT1/3, p38/STAT1, CXCL12/CXCR4, JAK/STAT, RAS/RAF/mTOR, and TGF-β1/TGF-β1R (Darvin et al., 2017; A Youness et al., 2021). Triterpenoids have been shown to suppress cancer progression by inhibiting NF-κB and STAT3 signaling pathways, downregulating Cyclin D1 expression, and inducing apoptosis, as reported in both natural and synthetic derivatives (Probst et al., 2015; Naz et al., 2020; Yadav et al., 2010). Evidence from previous studies suggests that these secondary metabolites exert anticancer effects through the suppression of cell proliferation and induction of apoptosis.

3.2. Cytotoxicity of *Bauhinia purpurea* leaf extracts

The cytotoxic activity of *Bauhinia purpurea* leaf extracts obtained using 96% ethanol, ethyl acetate, and n-hexane was evaluated against MCF-7 breast cancer cells through the MTT assay. This colorimetric method evaluates cell viability through the enzymatic activity of mitochondria, which reduce tetrazolium salts into purple formazan crystals, with the absorbance intensity reflecting the number of viable cells (Nurdiani et al., 2023).

Table 1
Phytochemical screening results of Bauhinia purpurea leaf extracts using 96% ethanol, ethyl acetate, and n-hexane.

Compound	96% Ethanol Extract	Ethyl Acetate Extract	n-Hexane Extract
Alkaloids	–	–	+
Polyphenols	+	–	+
Tannins	+	–	–
Flavonoids	+	+	–
Quinones	–	–	–
Monoterpenoids/Sesquiterpenoids	–	–	–
Steroids/Triterpenoids	+	+	+

Table 2
IC_{25}, IC_{50}, and IC_{75} doses from MTT assay results.

Extract	IC_{25} (µg/mL)	IC_{50} (µg/mL)	IC_{75} (µg/mL)
96% Ethanol	716.1602	979.9851	1252.5572
Ethyl Acetate	12.8172	88.5360	376.7887
n-Hexane	64.1803	271.8535	731.2728

In this research, the *Bauhinia purpurea* extract prepared with 96% ethanol was classified as non-cytotoxic (IC_{50} = 979.99 µg/mL), the ethyl acetate extract exhibited moderate cytotoxicity (IC_{50} = 88.54 µg/mL), and the n-hexane extract demonstrated weak cytotoxicity (IC_{50} = 271.85 µg/mL), as shown in Table 2. In addition, Figures 1–3 illustrate morphological alterations in MCF-7 cells consistent with apoptotic changes following treatment with these extracts.

The observed cytotoxic effects were likely influenced by solvent polarity, the solubility and concentration of active compounds, and potential interactions among bioactive molecules. Differences in IC_{50} values might be explained by variations in solvent polarity, which subsequently influence the efficiency of extraction and extraction yield of bioactive metabolites (Prasetya et al., 2021; Widyanto et al., 2020)."

Despite ethanol's ability to extract anticancer phytochemicals, the cytotoxicity assay results indicated that the ethanol extract was classified as non-cytotoxic. This apparent paradox may be attributed to antagonistic interactions among compounds within the crude extract, which could mask the activity of more potent constituents (Caesar et al., 2019; Rajčević et al., 2022). Such interactions in plant extracts can either enhance or diminish bioactivity; in some cases, purification increases cytotoxicity, whereas in others it reduces overall activity. The ethanol extract may also contain low-potency flavonoids or compounds that interfere with the function of more active molecules. Furthermore, certain phytochemicals may reduce toxicity by neutralizing the harmful effects of other constituents, thereby lowering the overall cytotoxic potential (Caesar et al., 2019).

It is also possible that the ethanol extract contains flavonoid types with low potency or lacking cytotoxic activity against MCF-7 cells. Previous studies have demonstrated that not all flavonoids exert the same degree of cytotoxicity. For instance, quercetin, luteolin, kaempferol, and chrysin exhibit strong antiproliferative effects, whereas genistein, naringenin, and daidzein display weak or no cytotoxic activity in MCF-7 cells. Myricetin and catechins have also been reported to exhibit no cytotoxicity among multiple breast cancer cell lines

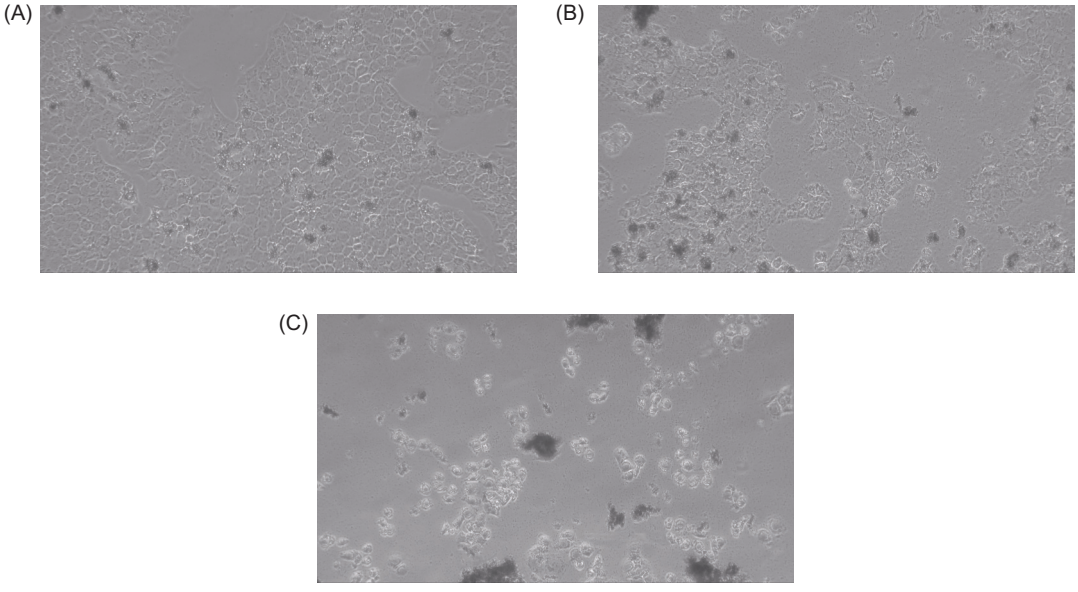


Figure 1. Morphology of MCF-7 cells treated with 96% ethanol extract at (A) IC_{25}, (B) IC_{50}, and (C) IC_{75} doses (Obj. 100x).

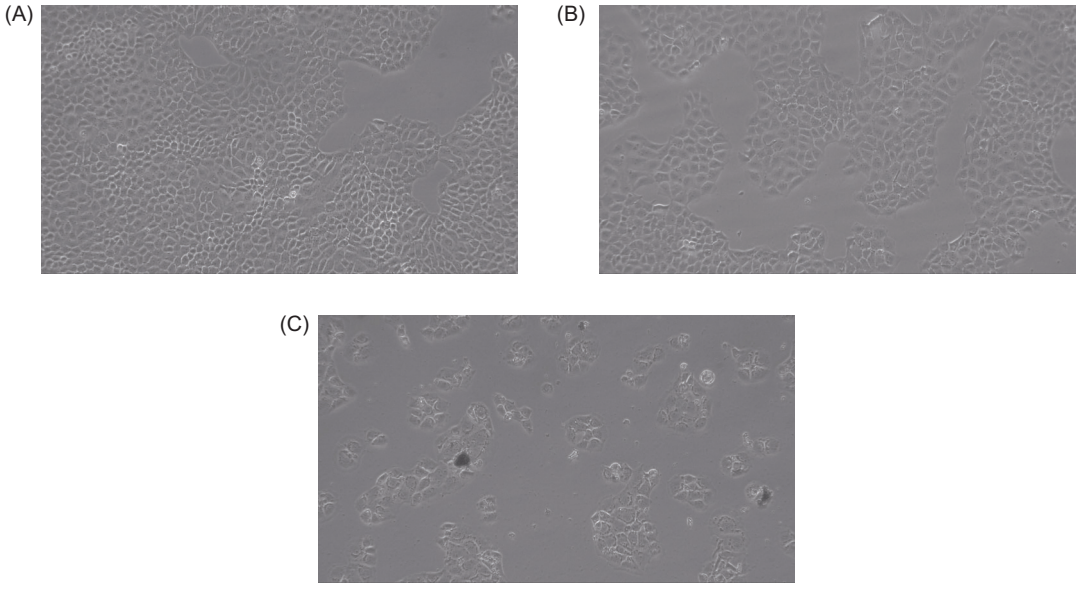


Figure 2. Morphology of MCF-7 cells treated with ethyl acetate extract at (A) IC_{25}, (B) IC_{50}, and (C) IC_{75} doses (Obj. 100x).

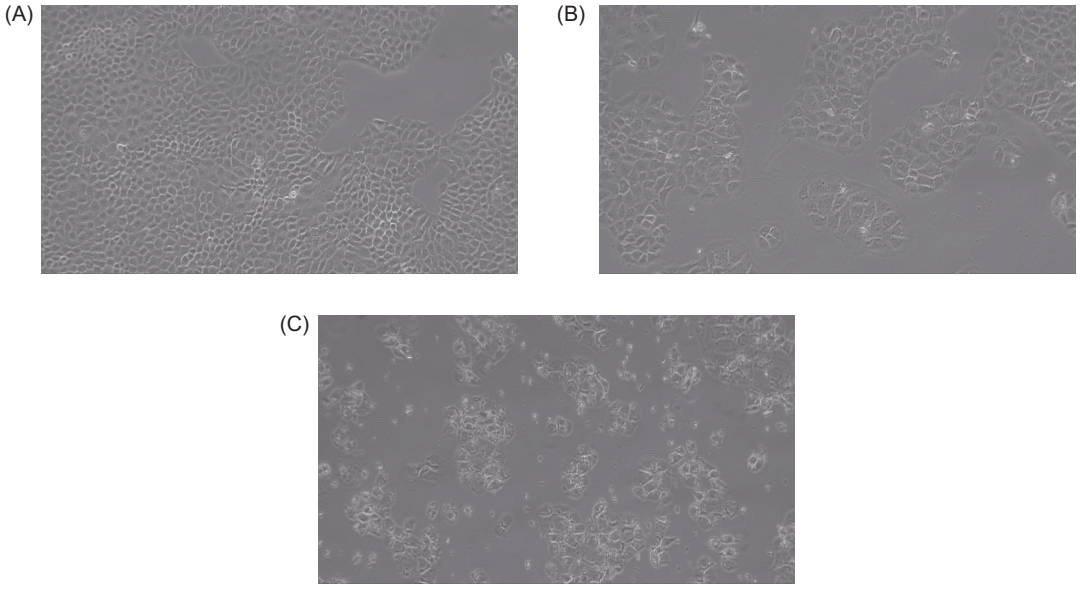


Figure 3. Morphology of MCF-7 cells treated with n-hexane extract at (A) IC_{25}, (B) IC_{50}, and (C) IC_{75} doses (Obj. 100x).

(Yadegarynia et al., 2012). In the present study, however, no further characterization was performed to determine the specific flavonoid constituents within the different extracts.

3.3. Relative expression of Cyclin D1 (*CCND1*) gene

Quantitative expression analysis revealed that all extracts significantly reduced CCND1 expression, demonstrating dose-dependent responses in MCF-7 cells. Relative to control (normalized to 1.00), ethanol, ethyl acetate, and n-hexane extracts decreased CCND1 expression, with the strongest suppression at IC_{75} concentrations. Doxorubicin showed a similar reduction (Table 3, Figures 4–5).

Statistical analysis using one-way ANOVA indicated significant differences among the groups ($p = 0.001$). Subsequent Tukey's post hoc test confirmed that CCND1 expression was significantly reduced in all treatment groups compared with the negative control ($p < 0.05$).

Phytochemical screening in this study revealed that flavonoids were present in the ethanol and ethyl acetate extracts of *Bauhinia purpurea* leaves, while steroids and triterpenoids

were detected in all extracts, including n-hexane. Several flavonoid, steroid, and triterpenoid compounds found in *Bauhinia purpurea* leaves have been reported to play a role in modulating the expression of *CCND1*,

Based on a 1987 study (as cited in Spilková & Húbik, 1992, and later referenced by Salatino et al., 1999), several flavonoids, including quercetin, quercitrin, apigenin, apigenin-7-O-glucoside, and rutin, have been identified in the leaves of *Bauhinia purpurea*. Notably, quercetin and apigenin are reported to inhibit the MAPK/ERK signaling pathway, which regulates transcription factors such as AP-1 that promote CCND1 expression (Woo et al., 2020; Silva et al., 2025). Moreover, Park et al. (2019) demonstrated that intraperitoneal quercetin treatment in mice significantly reduced CCND1 expression, in parallel with decreased activities of MAPK, ERK1/2, and AKT signaling pathways.

MAPK/ERK signaling is a key cascade regulating proliferation, differentiation, survival, and cellular adaptation. It includes three kinase levels: Ras, Raf (MAP3K), and MEK. Upon stimulation by growth factors, the intracellular Ras protein becomes activated, leading to sequential activation of Raf kinase and MEK1/2. MEK1/2 then phosphorylates ERK1/2, which translocates from the cytoplasm into the nucleus. Within the nucleus, ERK1/2 phosphorylates and activates transcription factors such as AP-1 (comprising c-Jun and c-Fos) and Ets, which bind specifically to the promoter region of the *CCND1* gene. This binding facilitates transcriptional upregulation of Cyclin D1, linking extracellular growth signals to cell cycle progression (Barbosa, 2021; Guo et al., 2020; Roskoski, 2012).

Quercetin has been reported to inhibit the PI3K/Akt/mTOR signaling pathway, a central regulator of cancer cell survival, proliferation, and therapy resistance. Cyclin D1 acts as a primary downstream effector of this pathway. Upon activation, PI3K catalyzes the conversion of PIP2 to PIP3, leading to recruitment and activation of Akt. Activated Akt promotes CCND1 transcription and protein expression while simultaneously inhibiting apoptotic signaling (Asgharian et al., 2022; Jiang et al., 2024; Rascio et al., 2021). Under normal physiological conditions, Akt supports cyclin D1 expression by stabilizing its mRNA and protein through suppression of GSK-3β-mediated degradation (Shimura et al., 2012). In cancerous cells, hyperactivation of Akt amplifies CCND1 transcription and enhances protein stability, thus facilitating continuous cell cycle progression and promoting

Table 3
Mean relative expression ratio of *CCND1* gene across different treatment groups.

Group	Mean	SD	Median	Min	Max	Range
Negative control	1.00	0.00	1.00	1.00	1.00	0.00
Ethanol IC_{25}	0.24	0.38	0.02	0.02	0.68	0.66
Ethanol IC_{50}	0.11	0.19	0.01	0.01	0.33	0.33
Ethanol IC_{75}	0.01	0.01	0.01	0.01	0.03	0.02
Ethyl acetate IC_{25}	0.30	0.11	0.33	0.18	0.40	0.22
Ethyl acetate IC_{50}	0.12	0.09	0.15	0.02	0.19	0.17
Ethyl acetate IC_{75}	0.04	0.02	0.05	0.02	0.05	0.03
n-hexane IC_{25}	0.26	0.30	0.14	0.05	0.60	0.55
n-hexane IC_{50}	0.23	0.19	0.28	0.01	0.39	0.38
n-hexane IC_{75}	0.10	0.15	0.02	0.01	0.28	0.27
Doxorubicin IC_{25}	0.25	0.35	0.08	0.03	0.66	0.63
Doxorubicin IC_{50}	0.20	0.27	0.09	0.01	0.50	0.50
Doxorubicin IC_{75}	0.10	0.14	0.03	0.00	0.25	0.25

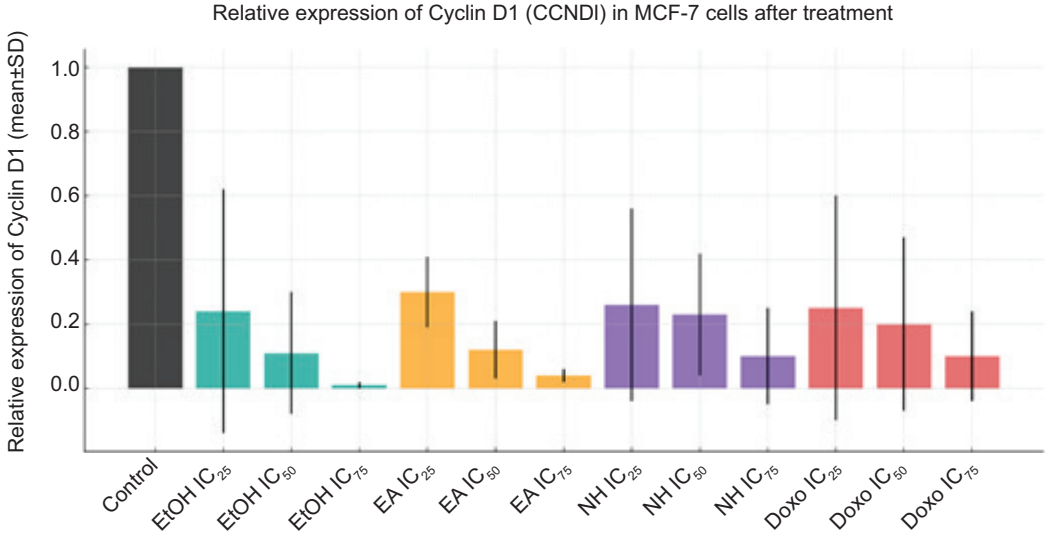


Figure 4. Average relative expression ratio of cyclin D1 (*CCND1*) gene in various treatment groups.

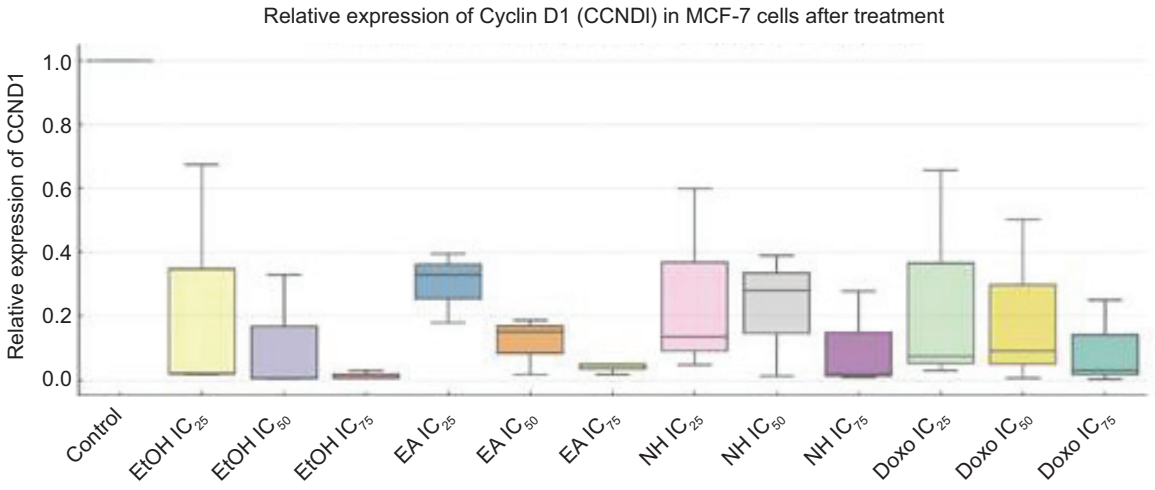


Figure 5. Box plot of relative expression of Cyclin D1 (*CCND1*) gene in MCF-7 cells treated with *Bauhinia purpurea* leaf extracts (96% ethanol, ethyl acetate, and n-hexane) and doxorubicin at IC25, IC50, and IC75 concentrations. The plot displays median, interquartile range, and variability across triplicate samples (n = 3 per group).

tumor growth. By inhibiting this pathway, quercetin may reduce cyclin D1 levels, disrupt cell cycle progression, and induce antiproliferative effects in malignant cells.

According to Turktekin et al. (2011), administration of apigenin significantly lowered CCND1 mRNA levels in HT29 colorectal cancer cells. The suppression of CCND1 transcription was associated with inhibition of cell cycle progression and activation of apoptotic pathways. These results indicate that apigenin mediates its anticancer activity, at least partially, through downregulation of CCND1 expression and interference with cell cycle progression. Xu et al. (2016) reported that the anticancer activity of apigenin in colorectal cancer cells involves inhibition of the Wnt/β-catenin signaling pathway. Specifically, apigenin blocks nuclear translocation of β-catenin, which is essential for activating transcription factors including TCF/LEF that regulate CCND1 expression. The findings of the study demonstrated that treatment with apigenin led to a decrease in the protein levels of both β-catenin and CCND1 in colorectal cancer cells. This reduction suggests that apigenin effectively suppresses the Wnt/β-catenin signaling pathway, which is critical for the regulation of cell cycle progression and proliferation. Furthermore, experiments employing β-catenin knockdown confirmed that the observed downregulation of CCND1 by apigenin is mediated specifically through inhibition of β-catenin signaling. These results collectively indicate that apigenin exerts its anticancer effects, at least in part, by targeting the β-catenin/CCND1 axis, thereby disrupting the transcriptional regulation of Cyclin D1 and impeding tumor cell proliferation.

Phytosterols, particularly β-sitosterol, have been identified as bioactive components in the *Bauhinia purpurea* leaves (Verma et al., 2009). Kan et al. (2025) demonstrated that β-sitosterol downregulates FGFR1 and EGFR, two tyrosine kinase receptors known to activate the PI3K/AKT/mTOR signaling pathway, thereby reducing the expression of Cyclin D1 in A549 lung cancer cells. Since Cyclin D1 plays a pivotal role in the G1/S phase transition, this suppression suggests the importance of β-sitosterol as an antiproliferative agent.

In addition to β-sitosterol, *Bauhinia purpurea* leaves contain triterpenoids such as lupeol and stigmasterol (Negi et al., 2012). Lupeol, in particular, has been shown to block prostate cancer cell proliferation by targeting the Wnt/β-catenin signaling pathway (Saleem et al., 2009). This pathway regulates the transcription of multiple oncogenes, including Cyclin D1, c-Myc, and MMP-2. By preventing the nuclear translocation of the β-catenin–TCF complex, lupeol effectively decreases Cyclin D1 expression. Supporting evidence from melanoma studies further confirmed that lupeol reduces cell viability, induces apoptosis, and suppresses the expression of Cyclin D1 and other proliferation markers (Tarapore et al., 2010).

The current findings indicate that *Bauhinia purpurea* leaf extracts are associated with a reduction in *CCND1* gene expression in MCF-7 cells, which may occur in a dose-dependent manner. This modulation could involve multiple signaling pathways, including MAPK/ERK, PI3K/AKT/mTOR, and Wnt/β-catenin, which regulate Cyclin D1 transcription. Phytoconstituents such as quercetin, apigenin, β-sitosterol, and lupeol may contribute to this effect by influencing cell cycle regulation and proliferation.

STUDY LIMITATIONS AND FUTURE DIRECTIONS

A limitation of this research is that the active compounds directly mediating cytotoxicity and *CCND1* gene modulation were not specifically identified or quantified. Crude extracts consist of complex combinations of bioactive molecules, including flavonoids, tannins, polyphenols, alkaloids, and triterpenoids, which were neither purified nor standardized. Consequently, it is difficult to ascertain which constituents are primarily responsible for the observed effects, and the biological activity may reflect synergistic, antagonistic, or predominant effects of one or more components.

Future studies should aim to isolate, purify, and characterize individual phytochemicals from *Bauhinia purpurea* leaf extracts to clarify their specific roles in mediating cytotoxicity and regulating CCND1 expression. Additional research is required to elucidate the molecular mechanisms involved, particularly those associated with the MAPK/ERK, PI3K/Akt, and Wnt/β-catenin signaling pathways. Further cytotoxicity assessments using diverse cancer cell lines are necessary to evaluate the potential selective activity of *Bauhinia purpurea* leaf extracts. Finally, in vivo investigations are crucial to confirm the therapeutic efficacy and safety of the active compounds identified in these extracts within relevant biological systems, thereby supporting their potential development as phytopharmaceutical agents.

CONCLUSIONS

Bauhinia purpurea leaf extracts exhibited varying cytotoxic activities depending on the solvent used: the ethyl acetate extract showed moderate cytotoxicity, the n-hexane extract was weakly cytotoxic, and the 96% ethanol extract was non-cytotoxic. All three extracts significantly downregulated cyclin D1 (*CCND1*) gene expression at IC_{25}, IC_{50}, and IC_{75} doses, with effects comparable to doxorubicin. These findings support the potential of *Bauhinia purpurea* as a phytopharmaceutical candidate for breast cancer treatment through cell cycle inhibition.

ETHICAL APPROVAL

The study protocol received approval from the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Indonesia (Approval Number: 391/UN4.6.4.5.31/PP36/2024; Protocol Number: UH24050294).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this study.

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AUTHOR CONTRIBUTIONS

Teja Koswara: Research concept and design, Collection and/or assembly of data, Data analysis and interpretation, Writing the article; Rina Masadah: Research concept and design, Data analysis and interpretation; Berti Julian Nelwan: Research concept and design, Data analysis and interpretation; Prihantono: Critical revision of the article; Andi Dian Permana: Critical revision of the article; Andi Alfian Zainuddin: Critical revision of the article; Primariadewi Rustamadji: Critical revision of the article.

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As shown in Figures 1–3, morphological examination revealed that MCF-7 breast cancer cells treated with *Bauhinia purpurea* leaf extracts (96% ethanol, ethyl acetate, and n-hexane) at various concentrations for 48 hours exhibited characteristic apoptotic features, including cell rounding, membrane swelling, and loss of intercellular adhesion.

Figure 4 illustrates the mean gene expression levels of CCND1 in MCF-7 breast cancer cells following treatment with *Bauhinia purpurea* leaf extracts (96% ethanol, ethyl acetate, and n-hexane) at IC25, IC50, and IC75 doses, compared to untreated control and doxorubicin-treated cells. A dose-dependent decrease in CCND1 expression was observed, with the highest suppression seen at IC75 doses.

As illustrated in Figure 5, all treatment groups showed a reduction in CCND1 expression compared to the control. Ethanol and ethyl acetate extracts showed the most consistent and pronounced suppression at IC75, with median values below 0.05 and minimal variability. In contrast, the n-hexane extract displayed greater variation, particularly at lower doses. Doxorubicin produced comparable effects at IC75, confirming dose-dependent downregulation of Cyclin D1 expression.