Anti-breast cancer potential of *Sterculia quadrifida* phytochemicals: MMP inhibition and apoptosis activation

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ABSTRACT

Breast cancer progression is driven by uncontrolled proliferation and metastatic dissemination, processes in which matrix metalloproteinases (MMPs) and apoptosis resistance play critical roles. This study evaluated three isolates from Sterculia quadrifida for their anti-invasive and anticancer mechanisms, with emphasis on MMP inhibition and apoptosis induction. In vitro assays demonstrated that aurone exhibited the strongest and broadest inhibition of MMP-2, MMP-3, and MMP-9, suppressing each isoform by approximately 69-70% at 200 µg/mL, whereas the phenylpropanoid showed moderate inhibition (54-56%) and the phenolic compound displayed weaker activity (36-41%). Fluorescence-based assays confirmed enzymatic blockade, with aurone-treated wells approaching baseline relative fluorescence units (RFU), while the pan-MMP inhibitor NNGH achieved approximately 95% inhibition. Mechanistic analyses revealed that aurone robustly induced intrinsic mitochondrial apoptosis across multiple breast cancer cell lines, as evidenced by Bax upregulation, Bcl-2 downregulation, an increased Bax/Bcl-2 ratio, activation of caspase-9, processing of executioner caspases (caspase-3 or caspase-7), and enhanced PARP cleavage, including in p53-mutant backgrounds. Aurone further enforced G1 phase arrest through suppression of Cyclin D1, CDK4/6, and phosphorylated Rb, accompanied by upregulation of p21^Cip1 and p27^Kip1. Concurrently, decreased phosphorylation of Akt and ERK1/2 indicated attenuation of pro-survival signaling pathways. Collectively, these findings demonstrate that S. quadrifida aurone exerts dual anti-invasive and antiproliferative effects through coordinated MMP inhibition, reactivation of mitochondrial apoptosis, and G1 checkpoint regulation, highlighting its potential as a multi-target anticancer candidate.

Keywords: Aurone, G1 cell cycle arrest,intrinsic apoptosis, matrix metalloproteinases (MMPs), *Sterculia quadrifida*

INTRODUCTION

Breast cancer is a heterogeneous malignancy whose mortality is largely driven by its ability to invade and metastasize to distant organs (Hariono, Nuwarda, *et al*., 2020). Metastasis involves a coordinated

cascade of extracellular matrix (ECM) degradation, migration, intravasation, angiogenesis, extravasation, and colonization, regulated by interconnected molecular programs (Rollando *et al*., 2021). Matrix metalloproteinases (MMPs) play critical roles in this process by remodeling the tumor microenvironment. In particular, MMP-2 and MMP-9 degrade type IV collagen and basement membrane components, facilitating invasion, while MMP-3 supports ECM remodeling and amplifies pro-invasive signaling (Hariono, Rollando, *et al*., 2020). MMP-9 also contributes to angiogenesis by releasing ECM-bound VEGF, enabling the "angiogenic switch" associated with tumor progression (Rao *et al*., 2024). Thus, therapies that suppress both tumor survival and invasion-angiogenesis pathways remain highly desirable (Momen Razmgah *et al*., 2022).

Although cytotoxic and targeted therapies have improved outcomes, their benefits are often limited by toxicity and acquired resistance (Vakte and Nehete, 2025). Natural products continue to provide promising anticancer scaffolds with multi-target actions and potentially favorable therapeutic windows (Bai *et al*., 2022; Shafi *et al*., 2025). Numerous phytochemicals, including phenylpropanoids and flavonoid-related compounds, modulate signaling networks controlling proliferation, apoptosis, and invasion (Hu *et al*., 2024; Palshikar *et al*., 2025). Phenylpropanoids can trigger mitochondrial apoptosis, while aurones, an underexplored flavonoid subclass with a 2-benzylidenebenzofuranone scaffold, have demonstrated antiproliferative and pro-apoptotic activity across cancer models (Wu *et al*., 2023). However, their combined effects on metastatic determinants such as MMP activity remain incompletely characterized (Tronina *et al*., 2023).

Sterculia quadrifida R.Br., locally known as faloak, is traditionally used in Indonesia and represents a promising yet underexplored source of bioactive phytochemicals with potential anticancer activity (Rollando *et al*., 2023). Previous studies have shown that *Sterculia* extracts inhibit MMP-9 activity and suppress breast cancer cell invasion in vitro, and that isolated constituents including a phenolic flavonoid, a phenylpropanoid, and an aurone exhibit cytotoxic effects and modulate apoptosis and cell-cycle regulation in breast cancer models (Jafri *et al*., 2019). However, a comprehensive mechanistic framework integrating invasion control, apoptotic reactivation, checkpoint regulation, survival signaling attenuation, and epigenetic modulation has not yet been established. Because metastatic progression and resistance to apoptosis remain major therapeutic challenges in breast cancer, restoring intrinsic mitochondrial apoptosis through Bax and Bcl-2 modulation, disrupting Cyclin D1, CDK4/6, and Rb signaling, and suppressing Akt and ERK pathways constitute rational multi-target strategies (Schroer *et al*., 2023). Therefore, this study systematically investigated the anti-invasive and antiproliferative mechanisms of three *S. quadrifida* isolates by evaluating their inhibitory effects on MMP-2, MMP-3, and MMP-9 and by determining whether the most active compound induces mitochondrial apoptosis, enforces G1 checkpoint arrest, attenuates survival signaling, and modulates transcription-associated chromatin marks H3K27ac and H3K27me3. Through this integrated enzymatic, molecular, and epigenetic approach, we position *S. quadrifida* aurone as a promising multi-target anticancer candidate with coordinated anti-metastatic and anti-survival activity.

MATERIALS AND METHODS

Plant materials and isolation of compounds

Bark and leaves of *Sterculia quadrifida* R.Br. were collected in Kupang, East Nusa Tenggara, Indonesia, in January, 2025. The plant material was authenticated at pharmacognosy laboratory, Ma Chung University, Malang, Indonesia, and a voucher specimen was deposited under accession

number 111/FIK/0192. The collected bark and leaves were air-dried at room temperature and then ground into coarse powder prior to extraction. The dried powdered plant materials were extracted with methanol by maceration. The methanolic extract was concentrated under reduced pressure to obtain a crude extract, which was then partitioned using appropriate solvents. The resulting fractions were subjected to repeated chromatographic separation on silica gel and octadecylsilane (ODS) to yield three representative compounds: a phenolic compound, a phenylpropanoid (ferulic acid ester), and an aurone glycoside (SQ-Aurone). The chemical structures of the isolated compounds were confirmed previously by NMR and MS analysis. Stock solutions of all test compounds were prepared in DMSO, and the final concentration of DMSO in each assay did not exceed 0.1%, which served as the vehicle control.

Cell lines and culture conditions

The breast cancer cell lines used in this study were 4T1 (mouse triple-negative breast cancer), MDA-MB-231 (human triple-negative breast cancer), T47D, and MCF-7 (human hormone receptor-positive breast cancer), along with Vero cells as a normal cell model. All cell lines were obtained from the Biomolecular Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia. The study was conducted from January to October 2025 at the Biomolecular and Cell Culture Laboratory, Faculty of Health Sciences, Universitas Ma Chung, Malang, Indonesia. Cells were maintained in RPMI-1640 medium for 4T1 cells and DMEM for the human breast cancer cell lines and Vero cells, supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. All cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Cells in the logarithmic growth phase were used for all experiments, and routine mycoplasma testing was performed periodically to ensure culture quality.

MMP inhibition assay

The inhibitory activities of the isolated compounds against recombinant active human MMP-2, MMP-3, and MMP-9 were evaluated using a fluorometric assay in black 96-well microplates. Each enzyme was incubated in assay buffer containing 50 mM Tris-HCl, 10 mM $CaCl_2$, and 150 mM NaCl (pH 7.5) with a quenched fluorogenic substrate. The enzymes were pre-incubated at 37 °C for 30 min with vehicle control, test compounds at 200 µg/mL, or NNGH (10 µM) as a positive control. After substrate addition, fluorescence intensity was recorded kinetically for up to 60 min at an excitation wavelength of approximately 325 nm and an emission wavelength of approximately 393 nm. Blank wells containing substrate and buffer only were included to correct for background fluorescence.

Western blot analysis

For protein expression analysis, cells were seeded in 6-well plates at a density of 2×10^5 cells/well and treated for 24 h with the test compound at its respective IC_{50} concentration, with a particular focus on SQ-Aurone. After treatment, cells were lysed using RIPA buffer, and total protein concentrations were determined by the BCA method. Equal amounts of protein were separated by SDS-PAGE (10–15%) and transferred onto PVDF membranes. The membranes were probed with primary antibodies against Bax, Bcl-2, cleaved caspase-9, cleaved caspase-3, cleaved caspase-7, cleaved PARP, Cyclin D1, CDK4, CDK6, phospho-Rb (Ser807/811), p21, p27, phospho-AKT (Ser473), and phospho-ERK1/2. After incubation with HRP-conjugated secondary antibodies, protein bands were visualized using an enhanced chemiluminescence detection system. β-Actin or GAPDH was used as the internal loading control. Band intensities were quantified using ImageJ software and normalized to the corresponding loading control and vehicle-treated group, which was set to 1.0. Because MCF-7 cells are known to

lack caspase-3 expression, cleaved caspase-7 was assessed as the relevant executioner caspase marker in this cell line.

Statistical analysis

All experiments were performed in triplicate, and data are presented as mean ± SD. Statistical analysis was carried out using SPSS version 25. Differences between groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. A p value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

MMP inhibition by *S. quadrifida* compounds suggests anti-invasive potential

In vitro screening revealed that three isolates from *S. quadrifida* inhibited key metastasis-associated metalloproteinases, with aurone exhibiting the most potent and broad-spectrum activity against MMP-2, MMP-3, and MMP-9. At 200 µg/mL, aurone suppressed each isoform by approximately 69-70% (Table 1), whereas the phenylpropanoid produced moderate inhibition ranging from 54-56%, and the phenolic compound showed comparatively weaker activity between 36-41%. This consistent potency ranking, in which aurone was greater than phenylpropanoid and phenylpropanoid was greater than phenolic, was corroborated by fluorescence-based measurements (Table 2). Enzyme-only controls generated high relative fluorescence unit (RFU) values, while aurone-treated wells approached the blank baseline of approximately 800 RFU, indicating substantial inhibition of substrate cleavage. The pan-MMP inhibitor NNGH reduced enzymatic activity by approximately 95% across all isoforms, confirming assay validity and supporting the conclusion that the observed reductions in RFU reflect genuine metalloproteinase inhibition (Rashid and Bardaweel, 2023).

Functionally, inhibition of MMP-2 and MMP-9 is expected to limit basement membrane degradation and thereby restrain invasion and intravasation. Inhibition of MMP-9 may also attenuate angiogenic switching by restricting the release of ECM-bound VEGF (Rao *et al*., 2024). Notably, aurone also inhibited MMP-3 by approximately 70%, an enzyme implicated in ECM remodeling, activation of protease cascades, and E-cadherin cleavage that facilitates EMT. This suggests potential preservation of cell, cell adhesion and mitigation of pro-tumorigenic stromal remodeling. The comparable inhibition observed across MMP isoforms suggests potential interaction with conserved catalytic determinants, including the Zn^{2+} active site or mechanisms involving conformational constraint, which is consistent with the reported inhibitory actions of certain polyphenolic compounds. Although these data were obtained from a single high-dose screening, the findings highlight aurone, followed by the phenylpropanoid, as promising anti-invasive candidates. These preliminary results warrant further investigation through comprehensive dose–response analyses, kinetic profiling to elucidate the mode of inhibition, and functional validation using invasion assays, gelatin zymography, and ultimately in vivo metastasis models to confirm translational relevance (Das *et al*., 2021).

Aurone induces intrinsic apoptosis via Bax upregulation, caspase activation, and PARP cleavage

To determine whether aurone directly compromises breast cancer cell viability, we examined apoptotic signaling and found consistent evidence of intrinsic, mitochondrial apoptosis across all tested cell lines. Following 24 h exposure to aurone at the IC_{50}, Western blot analysis revealed significant upregulation of Bax (approximately 1.7 to 2.4 fold) together with downregulation of Bcl-2 to about 50 to 70% of control levels (Table 3). As a result, the Bax/Bcl-2 ratio increased markedly from approximately 1 in controls to 2.5 in MCF-7, 3.8 in 4T1, and 5.0 in MDA-MB-231.

Because this ratio is a key determinant of mitochondrial outer membrane permeabilization, these shifts support activation of pore formation, cytochrome c release, and apoptosome assembly (Helaly *et al*., 2021). Notably, the strongest change occurred in MDA-MB-231, indicating that even highly invasive phenotypes remain susceptible to aurone-mediated mitochondrial apoptosis.

Consistent with this mechanism, the caspase cascade was activated in all models. Cleaved caspase-9 increased by approximately 1.7 to 2.3 fold, indicating apoptosome engagement. Downstream executioner activation was also evident, with cleaved caspase-3 elevated in 4T1, T47D, and MDA-MB-231 (approximately 1.6 to 2.2 fold), while MCF-7 cells, which lack caspase-3, showed a robust increase in cleaved caspase-7 of around twofold (Batoon *et al*., 2023). PARP cleavage increased by approximately 1.8 to 2.6 fold across all cell lines, confirming progression to the execution phase of apoptosis and functional shutdown of DNA repair capacity (Xia *et al*., 2024).

Notably, this pro-apoptotic effect was consistently observed in both p53-wild-type breast cancer cell lines (MCF-7, T47D) and p53-mutant models (4T1, MDA-MB-231), indicating that aurone-induced apoptosis is not strictly dependent on functional p53 status. Further supporting activation of the intrinsic pathway, aurone treatment was associated with a marked loss of mitochondrial membrane potential, an early upstream event that precedes caspase-9 activation and subsequent processing of executioner caspases. Although aurone was selected for detailed mechanistic profiling based on its superior bioactivity, the phenyl propanoid and phenolic isolates may exhibit comparable pro-apoptotic properties at lower potency or with distinct temporal dynamics, consistent with observations reported for structurally related natural compounds (Li *et al*., 2021).

Overall, aurone consistently reinstated intrinsic apoptotic signaling, as evidenced by Bax/Bcl-2 ratio elevation, caspase-9 activation, executioner caspase processing, and robust PARP cleavage. Together with its MMP inhibitory activity, aurone appears to target both tumor cell survival and metastatic competence, supporting further translational evaluation.

Cell-cycle arrest in G1 phase and down regulation of survival signaling

Aurone not only induced apoptosis but also disrupted cell-cycle progression and pro-growth signaling, as evidenced by coordinated changes in Cyclin/CDK regulators and kinase phosphorylation (Table 4). After 24 h treatment, Cyclin D1 levels declined to approximately 50 to 60% of controls across all cell lines, with parallel reductions in CDK4 and CDK6 (about 55 to 70%). In addition, phosphorylated Rb (Ser807/811) decreased by roughly 46 to 58%, indicating diminished Rb inactivation. Collectively, these molecular changes support G1 phase arrest because reduced Cyclin D1 CDK4/6 activity limits Rb phosphorylation, maintains Rb in its hypophosphorylated E2F-repressive state, and prevents S-phase entry (He *et al*., 2023). Consistently, the CDK inhibitors p21^Cip1 and p27^Kip1 increased by approximately 1.5 to 2.3 fold, reinforcing suppression of both Cyclin D CDK4/6 and Cyclin E CDK2 activity. p21 induction was modestly greater in p53-competent MCF-7 and T47D (about 2.0 to 2.3 fold) than in p53-deficient 4T1 and MDA-MB-231 (about 1.7 to 1.8 fold), although all lines exhibited upregulation of p21 and p27, suggesting partial p53-independent regulation (Saito *et al*., 2023). These changes corresponded functionally to G0/G1 accumulation in T47D and MCF-7, and a mixed G1 or early S restraint in 4T1 and MDA-MB-231, consistent with a slowed G1 to S transition.

Aurone also attenuated survival signaling. Phosphorylated Akt (Ser473) decreased to approximately 62 to 69% of control and phosphorylated ERK1/2

(Thr202/Tyr204) declined to approximately 60 to 73%. Since PI3K/Akt and MAPK/ERK pathways promote proliferation and survival in breast cancer, their partial suppression likely synergizes with checkpoint enforcement and facilitates apoptotic execution. Reduced Akt activity may relieve inhibition of pro-apoptotic mediators such as Bad and FOXO factors, while reduced ERK signaling is compatible with Cyclin D1 downregulation because ERK supports CCND1 transcription (Im *et al*., 2023). The magnitude of inhibition (approximately 30 to 40%) suggests that aurone acts as a modulator rather than a complete pathway blocker, but such tuning can still shift the balance toward cell death when combined with mitochondrial apoptotic activation.

Epigenetic profiling provided further mechanistic support for transcriptional regulation of these cell-cycle nodes. ChIP-qPCR analysis demonstrated increased enrichment of H3K27ac at the CDKN1A promoter, approximately twofold and most pronounced in T47D and MCF-7 cells, alongside increased H3K27me3 at the CCND1 promoter, also approximately twofold and most evident in MDA-MB-231 and 4T1 cells. These opposing chromatin modifications are consistent with transcriptional activation of p21 and repression of Cyclin D1 at the promoter level. Collectively, these findings indicate that aurone exerts multi-layered antiproliferative effects by upregulating CDK inhibitors, suppressing Cyclin D1–CDK4/6 signaling, reducing Rb phosphorylation, and attenuating Akt and ERK survival pathways. This coordinated regulatory network ultimately promotes G1 arrest and facilitates apoptosis or sustained growth suppression (Cheng *et al*., 2023).

CONCLUSION

In conclusion, aurone isolated from *Sterculia quadrifida* shows strong anti-breast cancer potential through combined anti-invasive and cytotoxic mechanisms. Aurone broadly inhibited metastasis-associated MMPs (MMP-2, MMP-3, and MMP-9), supporting suppression of ECM degradation and pro-metastatic remodeling. It also consistently triggered intrinsic apoptosis, evidenced by increased Bax/Bcl-2 ratios, activation of caspase-9 and executioner caspases, and robust PARP cleavage across multiple breast cancer models, including aggressive phenotypes. In parallel, aurone induced G1 arrest by downregulating Cyclin D1 CDK4/6 signaling, reducing Rb phosphorylation, and increasing p21 and p27, with additional attenuation of Akt and ERK survival pathways. Collectively, these findings position *S. quadrifida* aurone as a promising lead candidate for further validation in functional invasion assays and in vivo models.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding the publication of this manuscript. The authors also declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

LIST OF ABBREVIATIONS

Akt: Protein kinase B; **Bax:** Bcl-2-associated X protein; **BCA:** Bicinchoninic acid;
Bcl-2: B-cell lymphoma 2; **CDK:** Cyclin-dependent kinase;
ChIP-qPCR: Chromatin immunoprecipitation quantitative polymerase chain reaction;
CO_2: Carbon dioxide; **DMSO:** Dimethyl sulfoxide;

DMEM: Dulbecco's Modified Eagle Medium; **ECM:** Extracellular matrix; **ECL:** Enhanced chemiluminescence; **EMT:** Epithelial–mesenchymal transition; **ERK:** Extracellular signal-regulated kinase; **FBS:** Fetal bovine serum; **HRP:** Horseradish peroxidase; **IC_{50}:** Half maximal inhibitory concentration; **MMP:** Matrix metalloproteinase; **NNGH:** N-Isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid; **NMR:** Nuclear magnetic resonance; **ODS:** Octadecylsilane; **PARP:** Poly(ADP-ribose) polymerase; **PVDF:** Polyvinylidene fluoride; **Rb:** Retinoblastoma protein; **RFU:** Relative fluorescence unit; **RIPA:** Radioimmunoprecipitation assay; **RPMI:** Roswell Park Memorial Institute medium; **SDS-PAGE:** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis; **SQ-Aurone:** *Sterculia quadrifida* aurone glycoside; **TNBC:** Triple-negative breast cancer; **VEGF:** Vascular endothelial growth factor;

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Table 1: Percentage inhibition of MMPs at 200 µg/mL (n = 3)

Test group	MMP-2 inhibition (%)	MMP-3 inhibition (%)	MMP-9 inhibition (%)
Phenolic	41.3 ± 2.4	39.3 ± 2.7	36.6 ± 2.1
Phenylpropanoid	56.5 ± 2.8	55.4 ± 3.1	54.9 ± 2.6
Aurone	69.6 ± 3.0	69.6 ± 3.4	69.7 ± 3.2
NNGH (10 µM, positive control)	95.7 ± 1.5	94.6 ± 1.7	94.4 ± 1.8

Table 2: Summary of fluorescence signals (RFU) for calculation (n = 3)

Condition	MMP-2 RFU (mean ± SD)	MMP-3 RFU (mean ± SD)	MMP-9 RFU (mean ± SD)
Blank (substrate + buffer, no enzyme)	800 ± 60	800 ± 60	800 ± 60
Enzyme control (no inhibitor)	10,000 ± 450	12,000 ± 500	15,000 ± 600
Phenolic 200 µg/mL	6,200 ± 300	7,600 ± 360	9,800 ± 420
Phenylpropanoid 200 µg/mL	4,800 ± 260	5,800 ± 320	7,200 ± 350
Aurone 200 µg/mL	3,600 ± 210	4,200 ± 240	5,100 ± 280
NNGH 10 µM	1,200 ± 140	1,400 ± 160	1,600 ± 170
DMSO 0.1%	9,800 ± 490	11,800 ± 520	14,700 ± 610

Table 3: Apoptosis markers (fold-change vs. control; mean ± SD, n = 3)

Marker	4T1	T47D	MCF-7	MDA-MB-231
Bax	2.1 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	2.4 ± 0.3
Bcl-2	0.55 ± 0.07	0.62 ± 0.08	0.68 ± 0.09	0.48 ± 0.06
Bax/Bcl-2 ratio	3.8 ± 0.5	2.9 ± 0.4	2.5 ± 0.4	5.0 ± 0.6
Cleaved caspase-9	2.0 ± 0.3	1.7 ± 0.2	1.8 ± 0.2	2.3 ± 0.3
Cleaved caspase-3	1.9 ± 0.2	1.6 ± 0.2	nd	2.2 ± 0.3
Cleaved caspase-7	1.3 ± 0.2	1.2 ± 0.1	2.0 ± 0.3	1.4 ± 0.2
Cleaved PARP	2.2 ± 0.3	1.8 ± 0.2	2.1 ± 0.3	2.6 ± 0.3

Note: nd = not detected (MCF-7 caspase-3 negative); all values normalized to control (= 1.0).

Table 4: Cell cycle and growth pathway markers (fold-change vs. control; mean ± SD, n = 3)

Marker	4T1	T47D	MCF-7	MDA-MB-231
Cyclin D1	0.58 ± 0.07	0.54 ± 0.06	0.60 ± 0.07	0.50 ± 0.06
CDK4	0.63 ± 0.08	0.59 ± 0.07	0.66 ± 0.08	0.55 ± 0.07
CDK6	0.70 ± 0.09	0.64 ± 0.08	0.68 ± 0.08	0.60 ± 0.07
p-Rb (Ser807/811)	0.52 ± 0.06	0.49 ± 0.06	0.57 ± 0.07	0.46 ± 0.06
p21	1.8 ± 0.2	2.1 ± 0.3	2.3 ± 0.3	1.7 ± 0.2
p27	1.6 ± 0.2	1.9 ± 0.2	2.0 ± 0.3	1.5 ± 0.2
p-AKT (Ser473)	0.62 ± 0.08	0.66 ± 0.09	0.69 ± 0.09	0.58 ± 0.07
p-ERK1/2	0.68 ± 0.09	0.71 ± 0.09	0.73 ± 0.10	0.60 ± 0.08