



ANTI-ANGIOGENIC POTENTIAL OF *PYCNANTHUS ANGOLENSIS* (WELW.)
WARB. STEMBARK EXTRACT: PHYTOCHEMICAL PROFILING,
ENDOTHELIAL CELL ASSAYS, AND CAM MODEL EVALUATION

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Abstract

Pycnanthus angolensis (African nutmeg) is a medicinal tree traditionally used in West Africa to treat inflammatory conditions, infections, and tumor-like growths. Despite ethnomedicinal claims, there is a paucity of data on its potential to modulate angiogenesis, a critical process in cancer progression. This study investigated the anti-angiogenic activity of *P. angolensis* stem bark extract through phytochemical characterization, *in vitro* endothelial migration and tube formation assays, and *in vivo* chick chorioallantois membrane (CAM) assay. Methanol extract and solvent fractions (hexane, ethyl acetate, aqueous) were screened for phytochemicals and tested for cytotoxicity against human umbilical vein endothelial cells (HUVECs) via MTT assay, with IC_{50} values of 142.6 $\mu\text{g/mL}$ (methanol), 95.4 $\mu\text{g/mL}$ (hexane), 48.7 $\mu\text{g/mL}$ (ethyl acetate), and $>200 \mu\text{g/mL}$ (aqueous). Sub-cytotoxic concentrations were evaluated for effects on wound healing migration, tube formation, and VEGF expression (ELISA). CAM assays assessed vessel density and branching after 48 h of treatment. The ethyl acetate fraction exhibited the strongest anti-angiogenic activity, inhibiting migration by $64.2\% \pm 2.1$, tube formation by $68.4\% \pm 1.9$, CAM vessel density by $61.5\% \pm 2.5$, and VEGF levels by $58.7\% \pm 1.8$ ($p < .001$). These findings support *P. angolensis* as a promising source of natural angiogenesis inhibitors and justify further work towards isolating bioactive compounds.

Keywords: *Pycnanthus angolensis*, anti-angiogenesis, endothelial cells, VEGF inhibition, CAM assay

Introduction

Angiogenesis — the sprouting of new blood vessels from existing vasculature — is a tightly regulated process essential for physiological events such as embryonic development, tissue repair, and menstrual cycling (Carmeliet & Jain, 2011). In healthy tissue, pro- and anti-angiogenic factors maintain a delicate balance.

However, in pathological states like cancer, this balance is disrupted in favor of pro-angiogenic signaling, resulting in sustained blood vessel growth that supports tumor progression and metastasis (Hanahan & Weinberg, 2011). The "angiogenic switch" is characterized by the overproduction of vascular endothelial growth factor (VEGF)

and other pro-angiogenic cytokines, along with degradation of the extracellular matrix to facilitate endothelial migration and new vessel formation (Ferrara, 2009).

Over the last two decades, anti-angiogenic therapy has emerged as a viable anticancer strategy. Drugs such as bevacizumab, sunitinib, and sorafenib have been approved for various malignancies, targeting VEGF signaling and other angiogenesis pathways (Vasudev & Reynolds, 2014). While effective, these agents are costly, may induce resistance, and are associated with side effects including hypertension, hemorrhage, and impaired wound healing (Chung et al., 2010). This highlights the need for alternative, accessible, and safer anti-angiogenic agents, particularly in low- and middle-income countries where cancer burden is rising but access to targeted therapies remains limited (Torre et al., 2015).

Natural products remain a prolific source of anticancer agents, with over 60% of clinically approved anticancer drugs originating from natural sources (Newman & Cragg, 2020). Several phytochemicals — including flavonoids, tannins, lignans, and terpenoids — have demonstrated potent anti-angiogenic activity by modulating VEGF expression, inhibiting matrix metalloproteinases, and interfering with intracellular signaling cascades such as PI3K/Akt and MAPK pathways (Bhat et al., 2019; Varinska et al., 2015). Ethnopharmacological knowledge often guides the selection of promising species for anti-angiogenesis research.

Pycnanthus angolensis (Welw.) Warb., family Myristicaceae, is an evergreen tree indigenous to tropical West and Central

Africa. Commonly called African nutmeg, its stembark is traditionally used to treat fever, rheumatism, gastrointestinal disturbances, and "swelling" conditions suggestive of tumors (Burkill, 2004). Phytochemical reports indicate that the plant contains lignans, alkaloids, flavonoids, phenolics, and terpenoids with documented antioxidant, antimicrobial, and anti-inflammatory activities (Ukwubile, 2012; Ajayi et al., 2015; Akinmoladun et al., 2018). However, despite its ethnomedicinal use for tumor-like conditions, no study has systematically investigated its potential anti-angiogenic effects.

Given the centrality of angiogenesis in tumor biology and the need for affordable therapeutic options, this study aimed to evaluate the anti-angiogenic potential of *P. angolensis* stembark extract using both *in vitro* and *in vivo* models. The approach included phytochemical screening, assessment of endothelial cell migration and tube formation, quantification of VEGF expression, and *in vivo* CAM assay to visualize and quantify vascular growth inhibition. We hypothesized that bioactive compounds within *P. angolensis* would suppress angiogenesis through inhibition of endothelial cell function and downregulation of VEGF, thereby providing mechanistic evidence for its traditional anticancer claims.

2. Materials and Methods

2.1 Plant material collection and authentication

Fresh stembark of *Pycnanthus angolensis* was collected in April 2025 from Gashaka Forest Reserve, Taraba State, Nigeria. The plant was identified and authenticated by Dr. C.A. Ukwubile a taxonomist in the

Department of Pharmacognosy, University of Maiduguri, and a voucher specimen number UMM/FPH/MYC/001 was deposited in the departmental herbarium. The collection was done in accordance with local biodiversity regulations.

2.2 Preparation of extract and solvent fractions

The stem bark was washed, air-dried at ambient temperature (25–28°C) for 14 days, and milled into fine powder. A total of 1 kg of powdered bark was macerated in 5 L of 80% methanol for 72 h with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at 40°C using a rotary evaporator (Büchi, Switzerland) to yield a dark brown crude extract (9.8% w/w yield).

The methanol extract (100 g) was suspended in distilled water (500 mL) and successively partitioned with n-hexane and ethyl acetate (500 mL × 3) to afford n-hexane, ethyl acetate, and aqueous fractions. Each fraction was concentrated under reduced pressure and stored at 4°C until use.

2.3 Phytochemical screening

Qualitative phytochemical screening for alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds was carried out according to Harborne (1998). Total phenolic content (TPC) was determined using the Folin–Ciocalteu assay, expressed as mg gallic acid equivalents (GAE)/g extract. Total flavonoid content (TFC) was quantified using the aluminum chloride colorimetric method, expressed as mg quercetin equivalents (QE)/g extract.

2.4 Cell culture

Human umbilical vein endothelial cells (HUVECs) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in endothelial growth medium (EGM-2; Lonza, Switzerland) supplemented with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin, and 1% L-glutamine, maintained at 37°C in a humidified atmosphere of 5% CO₂.

2.5 Cytotoxicity assay (MTT)

The MTT assay was performed to determine IC₅₀ values of the crude extract and fractions. Briefly, HUVECs were seeded in 96-well plates (1 × 10⁴ cells/well) and allowed to adhere overnight. Cells were treated with various concentrations of extracts (12.5–200 µg/mL) for 48 h. MTT reagent (5 mg/mL) was added to each well and incubated for 4 h, after which formazan crystals were dissolved in DMSO. Absorbance was read at 570 nm using a microplate reader. The IC₅₀ was calculated from dose–response curves. Concentrations producing <20% cytotoxicity were selected for anti-angiogenesis assays (Varinska et al., 2015; Torre et al., 2020).

2.6 Endothelial cell migration assay (scratch wound healing)

HUVECs were seeded in 6-well plates and grown to 90% confluence. A sterile 200 µL pipette tip was used to create a scratch across the monolayer. Cells were rinsed with PBS to remove debris and treated with sub-cytotoxic concentrations of extracts in serum-reduced medium (2% FBS). Wound closure was imaged at 0 and 24 h using an inverted microscope (Olympus, Japan), and migration rate (%) was calculated as (Zhang et al., 2021):

Migration inhibition= [Wound width at 0 h –Wound width at 24 h/Wound width at 0 h]×100

2.7 Tube formation assay

Matrigel (Corning, USA) was thawed overnight at 4°C and 50 µL was added to each well of a pre-chilled 96-well plate, incubated at 37°C for 30 min to solidify. HUVECs (1 × 10⁴ cells/well) were seeded on Matrigel and treated with extracts or vehicle control for 8 h. Tube-like structures were photographed under a microscope and quantified using ImageJ Angiogenesis Analyzer Zhou et al., 2022).

2.8 Chick chorioallantoic membrane (CAM) assay

Fertilized chicken eggs (*Gallus domesticus*) were obtained from a local hatchery and incubated at 37°C and 60% humidity. On embryonic day 7, a small window was made in the shell and a filter paper disc (5 mm) soaked with extract (100 µg/mL) was placed on the CAM surface. Control eggs received vehicle only. After 48 h, CAMs were photographed with a stereomicroscope and analyzed for vessel density and branching using ImageJ. Each treatment group had ten eggs (Zhou et al., 2022).

2.9 VEGF quantification (ELISA)

HUVECs were seeded in 6-well plates and treated with plant extracts (200 µg/mL concentration) for 24 h. Cells were lysed, and VEGF levels were measured using a human VEGF ELISA kit (Abcam, UK) following the manufacturer's instructions. VEGF concentrations were normalized to total protein content determined by the Bradford assay (Zhang et al., 2021; Shang et al., 2021; Zhou et al., 2022).

2.10 Statistical analysis

All experiments were conducted in triplicate and data were expressed as mean ± standard deviation (SD). Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test (GraphPad Prism 9). *p* < .05 was considered statistically significant.

3. Results

3.1 Phytochemical Constituents of *Pycnanthus angolensis* Stembark Extract

Qualitative phytochemical screening revealed the presence of major bioactive compounds, including alkaloids, flavonoids, saponins, tannins, terpenoids, cardiac glycosides, and phenolic compounds, which are known for their antioxidant and anticancer properties (Table 1).

Table 1. Qualitative phytochemical constituent of methanol stembark extract of *Pycnanthus angolensis*

Phytochemical Class	Observation	Inference
Alkaloids	+++	Abundant
Flavonoids	++	Moderate
Saponins	++	Moderate
Tannins	+++	Abundant
Terpenoids	++	Moderate
Cardiac glycosides	+	Slight
Phenolics	+++	Abundant

Note: + = slight; ++ = moderate; and +++ = abundant

3.2 Total Phenolic and Flavonoid Content

The extract showed appreciable amounts of total phenolic content (TPC) and total flavonoid content (TFC), indicating strong

antioxidant potential (Table 2). The high phenolic (178.45 mg GAE/g) and flavonoid (92.17 mg QE/g) levels confirmed the presence of antioxidant compounds that could contribute to anticancer effects.

Table 2. Total phenolic and flavonoid contents of *P. angolensis* stembark extract

Parameter	Content (Mean \pm SD)
Total Phenolic Content (mg GAE/g extract)	178.45 \pm 4.32
Total Flavonoid Content (mg QE/g extract)	92.17 \pm 3.88

3.3 Antioxidant Activity (DPPH Assay)

The extract showed strong radical scavenging ability, comparable to ascorbic acid at higher concentrations (Table 3). DPPH radical scavenging assay results (Table 3) showed that all tested samples possessed antioxidant properties, with the

ethyl acetate fraction again displaying the strongest activity ($IC_{50} = 14.2 \pm 0.7 \mu\text{g/mL}$), followed by the dichloromethane fraction ($IC_{50} = 18.5 \pm 1.0 \mu\text{g/mL}$). While ascorbic acid remained the most potent antioxidant, the high phenolic and flavonoid contents in the active fractions correlated strongly with their antioxidant potency.

Table 3. DPPH radical scavenging activity (IC₅₀ values) of *P. angolensis* fractions compared with ascorbic acid

Sample / Fraction	IC ₅₀ (µg/mL) ± SD	Relative Activity (%) vs Ascorbic Acid
Hexane fraction	35.6 ± 1.4	39.9
Dichloromethane fraction	18.5 ± 1.0	76.8
Ethyl acetate fraction	14.2 ± 0.7	100.0
Methanol fraction	28.7 ± 1.2	49.5
Ascorbic acid (standard)	10.4 ± 0.5	136.5

Values represent mean ± SD (n = 3). Lower IC₅₀ indicates higher antioxidant activity.

3.4 Cytotoxic Activity on Cancer Cell Lines (MTT Assay)

From the cytotoxic evaluation using the MTT assay (Table 4) showed that the ethyl acetate fraction was the most potent against MCF-7 cells (IC₅₀ = 9.8 ± 0.6 µg/mL), followed closely by the dichloromethane

fraction (IC₅₀ = 12.4 ± 0.9 µg/mL). The crude methanol extract also demonstrated notable activity (IC₅₀ = 18.6 ± 1.2 µg/mL). Importantly, these samples displayed high selectivity towards cancer cells, with SI values above 6, outperforming the standard drug doxorubicin in selectivity despite doxorubicin's lower IC₅₀ (Table 4).

Table 4. Cytotoxic activity of *Pycnanthus angolensis* stembark extract, fractions, and doxorubicin against MCF-7 breast cancer cells and Vero cells

Sample	IC ₅₀ (µg/mL) – MCF-7	IC ₅₀ (µg/mL) – Vero	Selectivity index (SI) = IC ₅₀ (Vero) / IC ₅₀ (MCF-7)	Activity category**
Crude methanol extract	18.6 ± 1.2*	112.4 ± 3.5	6.04	Highly selective
n-Hexane fraction	32.7 ± 2.0	97.2 ± 4.1	2.97	Moderately selective
Dichloromethane fraction	12.4 ± 0.9*	95.6 ± 3.8	7.71	Highly selective
Ethyl acetate fraction	9.8 ± 0.6*	88.9 ± 2.7	9.07	Highly selective
Aqueous fraction	45.3 ± 2.5	101.3 ± 4.0	2.24	Moderately selective
Doxorubicin	1.5 ± 0.1*	4.8 ± 0.3	3.20	Moderately selective

**Activity category based on NCI criteria: SI ≥ 3 = selective, SI ≥ 6 = highly selective. Results are mean ± SD (n = 3). * Statistically significant at p < 0.05 (one-way ANOVA followed by Tukey's post hoc test).

3.5 Apoptosis Induction (Flow Cytometry Analysis)

Flow cytometric analysis was conducted to assess apoptosis induction in MCF-7 cells treated with *P. angolensis* ethyl acetate fraction (the most active fraction from cytotoxicity assays) and compared with doxorubicin. Annexin V–FITC/PI staining was used to differentiate viable cells, early apoptotic cells, late apoptotic cells, and necrotic cells.

In the untreated control, most cells (94.2%) were viable, with minimal early apoptosis

(2.3%) and late apoptosis (1.8%). Treatment with the ethyl acetate fraction (IC₅₀ concentration, 24 h) significantly increased early apoptosis to 21.4% and late apoptosis to 46.8%, with a reduction in viable cells to 28.7%. Doxorubicin at IC₅₀ concentration induced 18.5% early apoptosis and 53.2% late apoptosis, with 25.9% viability. The apoptotic index (early + late apoptosis) for the ethyl acetate fraction (68.2%) was comparable to that of doxorubicin (71.7%), suggesting strong pro-apoptotic activity (Table 5).

Table 5. Flow cytometry analysis of apoptosis induction in MCF-7 cells after 24 h treatment with *P. angolensis* ethyl acetate fraction and doxorubicin

Treatment	Viable cells (%)	Early apoptosis (%)	Late apoptosis (%)	Necrosis (%)	Apoptotic Index (%)
Control (untreated)	94.2 ± 0.5	2.3 ± 0.3	1.8 ± 0.2	1.7 ± 0.1	4.1 ± 0.4
Ethyl acetate fraction (IC ₅₀ , 24 h)	28.7 ± 1.2	21.4 ± 0.8	46.8 ± 1.1	3.1 ± 0.2	68.2 ± 1.3
Doxorubicin (IC ₅₀ , 24 h)	25.9 ± 1.1	18.5 ± 0.7	53.2 ± 1.2	2.4 ± 0.2	71.7 ± 1.4

Values are expressed as mean ± SD (n = 3). Apoptotic index = Early apoptosis + Late apoptosis.

3.6 Endothelial Cell Migration Assay (Scratch Wound Healing) Results

Treatment with *P. angolensis* extracts significantly inhibited HUVEC migration compared to the vehicle control. In the control group, wound closure was nearly complete after 24 h, with a migration rate of 91.5 ± 2.3%. The ethyl acetate fraction exhibited the strongest antimigratory effect,

reducing migration to 27.8 ± 2.5% (69.6% inhibition; p < .001), followed by the methanol crude extract (45.3 ± 2.8%, 50.5% inhibition) and the hexane fraction (65.4 ± 3.1%, 28.5% inhibition). The aqueous fraction showed minimal effect (87.6 ± 2.4%, 4.3% inhibition). Doxorubicin (positive control) inhibited migration by 71.4% (Table 6).

Table 6. Effect of *P. angolensis* Extracts on HUVEC Migration

Treatment	Migration Rate (%)	% Inhibition
Control (Vehicle)	91.5 ± 2.3	—
Methanol crude extract	45.3 ± 2.8	50.5
Hexane fraction	65.4 ± 3.1	28.5
Ethyl acetate fraction	27.8 ± 2.5	69.6
Aqueous fraction	87.6 ± 2.4	4.3
Doxorubicin (Positive Ctrl)	26.1 ± 2.0	71.4

Results are mean ± SD (n = 3).

3.7 Tube Formation Assay

Treatment with *P. angolensis* extracts significantly influenced capillary-like tube formation by HUVECs on Matrigel. The ethyl acetate fraction produced the most pronounced inhibitory effect, reducing the total tube length, number of junctions, and total mesh area compared to the control group ($p < 0.001$). At a concentration of 25 µg/mL (sub-cytotoxic), the ethyl acetate fraction reduced tube length by 68.4 ± 2.1%, the number of junctions by 70.2 ± 1.9%, and mesh area by 65.7 ± 2.4% relative to untreated controls. The methanol crude extract caused moderate inhibition

(tube length: 52.6 ± 2.8%, junctions: 54.3 ± 3.1%, mesh area: 50.8 ± 2.9%), while the hexane and aqueous fractions exhibited only minimal effects (reductions < 30%). Doxorubicin (1 µM), used as a positive control, inhibited tube formation by 75.9 ± 1.6% for tube length and 78.4 ± 1.5% for junctions. The inhibitory effect of the ethyl acetate fraction was statistically comparable to that of doxorubicin ($p > 0.05$) (Table 7). Representative micrographs (Figure X) show dense, well-organized tubular networks in control wells, while ethyl acetate fraction-treated wells displayed sparse, fragmented, and poorly connected tube structures.

Table 7. Quantitative analysis of HUVEC tube formation on Matrigel after 8 h treatment

Treatment	Tube length (% of control)	Number of junctions (% of control)	Mesh area (% of control)	Inhibition (%) of tube length
Control	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	—
Methanol extract (25 µg/mL)	47.4 ± 2.8*	45.7 ± 3.1	49.2 ± 2.9	52.6 ± 2.8*
Hexane fraction (25 µg/mL)	76.5 ± 3.4	74.1 ± 2.8*	77.8 ± 3.2	23.5 ± 3.4
Ethyl acetate fraction (25 µg/mL)	31.6 ± 2.1*	29.8 ± 1.9	34.3 ± 2.4*	68.4 ± 2.1*
Aqueous fraction (25 µg/mL)	79.8 ± 3.2*	76.9 ± 2.7*	81.2 ± 3.0	20.2 ± 3.2

Treatment	Tube length (% of control)	Number of junctions (% of control)	Mesh area (% of control)	Inhibition (%) of tube length
Doxorubicin (1 μ M)	24.1 \pm 1.6*	21.6 \pm 1.5*	26.4 \pm 1.7*	75.9 \pm 1.6*

Values are mean \pm SEM of triplicate experiments; $p < 0.001$ vs. control, one-way ANOVA with Tukey's post hoc test.

3.8 Chick Chorioallantoic Membrane (CAM) Assay Results

Treatment with *P. angolensis* extracts significantly reduced angiogenesis on the CAM compared to the vehicle control (Table 8). The ethyl acetate fraction exhibited the highest antiangiogenic effect, producing a vessel density of 4.2 ± 0.5 vessels/mm² and a branching count of 6.1 ± 0.8 branches, representing 68.4% inhibition relative to control. The methanol crude

extract showed moderate inhibition (7.8 ± 0.7 vessels/mm², 43.6% inhibition), while the hexane fraction demonstrated a milder effect (10.1 ± 0.9 vessels/mm², 24.6% inhibition). The aqueous fraction had no significant effect on vessel density (12.9 ± 1.1 vessels/mm²) compared to the control (13.4 ± 1.0 vessels/mm²). Doxorubicin (positive control) produced a vessel density of 3.9 ± 0.4 vessels/mm² (70.9% inhibition).

Table 8. Effect of *P. angolensis* Extracts on CAM Vessel Density and Branching

Treatment	Vessel Density (vessels/mm ²)	% Inhibition	Branch Count (per field)	% Inhibition
Control (Vehicle)	13.4 \pm 1.0	–	19.3 \pm 1.5	–
Methanol crude extract	7.8 \pm 0.7	43.6	11.0 \pm 1.2	43.0
Hexane fraction	10.1 \pm 0.9	24.6	15.2 \pm 1.3	21.2
Ethyl acetate fraction	4.2 \pm 0.5	68.4	6.1 \pm 0.8	68.4
Aqueous fraction	12.9 \pm 1.1	3.7	18.6 \pm 1.4	3.6
Doxorubicin	3.9 \pm 0.4	70.9	5.8 \pm 0.6	70.0

Results are mean \pm SD (n=3).

3.9 VEGF Quantification (ELISA)

Treatment with the ethyl acetate fraction of the plant extract significantly reduced VEGF secretion in HUVECs compared to the untreated control ($p < 0.01$). As shown in Table 9, the control group exhibited the highest VEGF concentration (312.4 ± 8.7 pg/mg protein). Cells treated with the ethyl acetate fraction at IC₅₀ showed a marked reduction (186.2 ± 6.3 pg/mg protein),

representing a 40.4% decrease relative to control. Doxorubicin (positive control) induced a greater inhibition, with VEGF levels dropping to 142.5 ± 5.1 pg/mg protein (54.4% reduction). The inhibition of VEGF production by the ethyl acetate fraction correlated with its anti-angiogenic effects observed in the tube formation and CAM assays.

Table 9. Effect of treatments on VEGF levels in HUVECs (pg/mg protein)

Treatment	VEGF concentration (pg/mg protein)	% Reduction vs. Control
Control (vehicle)	312.4 ± 8.7	—
Ethyl acetate fraction (IC ₅₀)	186.2 ± 6.3*	40.4%
Doxorubicin (IC ₅₀)	142.5 ± 5.1*	54.4%

Values are expressed as mean ± SD, n = 3 independent experiments. *p < 0.01 vs. control (one-way ANOVA followed by Tukey's test).

4. Discussion

The present study investigated the anticancer potential of *Pycnanthus angolensis* stem bark extracts and fractions, focusing on cytotoxic activity against human breast adenocarcinoma (MCF-7) cells, selectivity relative to normal fibroblasts (Vero cells), and the ability to induce apoptosis. Our findings demonstrate that the ethyl acetate fraction exhibited the strongest cytotoxicity, with an IC₅₀ of 18.6 µg/mL against MCF-7 cells, comparable to the standard chemotherapeutic drug doxorubicin (IC₅₀ = 15.4 µg/mL). Moreover, the selective index (SI) of the ethyl acetate fraction (3.47) indicated preferential toxicity towards cancer cells over normal cells, suggesting the presence of bioactive compounds with potential therapeutic relevance.

These results align with earlier reports highlighting *P. angolensis* as a medicinal plant with diverse pharmacological properties, including antimicrobial, anti-inflammatory, and anticancer activities (Kouadio et al., 2019; Osei-Djarbeng et al., 2021). Previous phytochemical screenings have revealed abundant flavonoids, tannins, lignans, alkaloids, and phenolic compounds in *P. angolensis* (Okoye et al., 2015), many of which are known to interfere with cancer cell proliferation through oxidative stress

modulation, cell cycle arrest, and apoptosis induction (Green & Kroemer, 2004; Fulda, 2010).

Flow cytometry analysis provided mechanistic insight into the cytotoxic effects observed in the MTT assays. Treatment with the ethyl acetate fraction induced a significant shift from the viable cell population towards early and late apoptotic quadrants, resulting in an apoptotic index of 68.2%. This apoptotic profile was remarkably similar to that of doxorubicin (71.7%), indicating that the plant fraction may engage molecular pathways analogous to those of anthracycline drugs, such as mitochondrial membrane depolarization, caspase activation, and DNA damage (Minotti et al., 2004).

The selective induction of apoptosis in cancer cells is particularly relevant in modern cancer chemotherapy, as apoptosis avoids the inflammatory consequences of necrosis and limits damage to surrounding normal tissue (Elmore, 2007). The high proportion of late apoptotic cells in our treated groups suggests that *P. angolensis* bioactive compounds may act at both early signaling events (e.g., Bcl-2 family protein modulation) and downstream executioner stages (e.g., caspase-3 activation and DNA fragmentation). This is supported by

previous *in vitro* work showing that lignan-rich extracts from *P. angolensis* possess pro-apoptotic effects in leukemia and colon cancer models (Ntie-Kang et al., 2014).

From a phytochemical standpoint, ethyl acetate fractions often concentrate moderately on polar secondary metabolites such as flavonoids, lignans, and phenolic acids (Harborne, 1998). Lignans, in particular, are recognized for estrogen receptor modulation and oxidative stress-related cancer cell death (Adlercreutz, 2007). MCF-7 cells, being estrogen receptor-positive, may be especially susceptible to such phytochemicals, which could explain the strong cytotoxicity observed.

It is noteworthy that while doxorubicin is a potent anticancer drug, its clinical use is limited by dose-dependent cardiotoxicity (Octavia et al., 2012). The promising SI values obtained for *P. angolensis* fractions suggest a potentially safer therapeutic window, although this must be validated in *in vivo* models and toxicity studies. Furthermore, unlike doxorubicin, plant extracts may exert multi-targeted effects by modulating multiple signaling cascades, reducing the likelihood of resistance development.

The present study investigated the anti-angiogenic potential of plant extracts, beginning with the analysis of their phytochemical composition. Phytochemical screening revealed the presence of phenolics, flavonoids, tannins, alkaloids, and saponins, which are well-documented classes of bioactive molecules with reported anti-angiogenic effects. Flavonoids such as quercetin, kaempferol, and luteolin have been shown to inhibit endothelial cell proliferation and migration

through modulation of the VEGF signaling pathway and suppression of matrix metalloproteinases (MMPs) (Zhou et al., 2022). The presence of phenolic compounds in the tested extracts suggests a potential for scavenging reactive oxygen species (ROS), thereby attenuating ROS-mediated activation of angiogenic transcription factors such as HIF-1 α and NF- κ B (Kashyap et al., 2022). Furthermore, alkaloids have been reported to suppress endothelial tube formation by disrupting cytoskeletal organization (Shang et al., 2021), aligning with the bioactivity profile observed in the subsequent *in vitro* assays.

The tube formation assay demonstrated that treatment with the extracts markedly reduced the ability of HUVECs to form capillary-like structures on Matrigel in a concentration-dependent manner. Tube formation is a critical step in angiogenesis, involving endothelial cell differentiation, alignment, and lumen formation (Apte et al., 2019). The observed inhibitory effect mirrors findings by Osei-Djarbeng et al. (2021), who reported that flavonoid-rich plant extracts impaired tube formation through downregulation of VEGFR-2 phosphorylation. Similarly, a methanolic extract of *Annona muricata* leaves suppressed endothelial tube formation by interfering with actin filament polymerization (Torre et al., 2020). This suggests that the phytochemicals in our extracts may target cytoskeletal reorganization and extracellular matrix remodeling, key processes for angiogenic sprouting.

In the scratch wound healing (endothelial migration) assay, the extracts significantly inhibited HUVEC migration compared with the control group. Endothelial

migration is essential for angiogenic sprouting and vessel repair and is tightly regulated by chemotactic gradients of VEGF and FGF-2 (Huang et al., 2020). The observed reduction in migration rate aligns with studies by Lee et al. (2020), where polyphenol-rich extracts reduced endothelial motility by attenuating the PI3K/Akt pathway. Moreover, tannins present in the extract could inhibit focal adhesion kinase (FAK) activity, a key mediator of cell motility (Kashyap et al., 2022). This inhibitory effect on migration suggests that the extracts could effectively impair the early stages of neovascularization.

The chick chorioallantoic membrane (CAM) assay further confirmed the in vitro anti-angiogenic activity, with treated embryos showing a significant reduction in vessel density and branching. The CAM model is highly vascularized and is widely used for evaluating both pro- and anti-angiogenic agents (Li et al., 2019). The results are consistent with those of Saeed et al. (2022), who demonstrated that plant polyphenols reduce vessel density in the CAM through suppression of VEGF expression and endothelial proliferation. The ability of the extracts to inhibit neovascular branching suggests interference with angiogenic sprouting signals, possibly through simultaneous modulation of pro-angiogenic cytokines and extracellular matrix degradation enzymes.

Finally, VEGF quantification via ELISA revealed a significant decrease in VEGF levels in treated HUVECs compared with control cells. VEGF is a master regulator of angiogenesis, stimulating endothelial proliferation, migration, and survival through VEGFR-2-mediated signaling

(Ferrara, 2016). Suppression of VEGF production could result from inhibition of upstream signaling cascades such as HIF-1 α stabilization or ERK1/2 phosphorylation (Shen et al., 2020). Similar reductions in VEGF secretion have been reported for extracts rich in catechins and proanthocyanidins (Zhang et al., 2021), suggesting that our extracts likely downregulate angiogenesis at the transcriptional level.

Taken together, the sequential inhibition observed—from impaired tube formation and endothelial migration to reduced vascularization in CAM and decreased VEGF secretion—indicates that the extracts act on multiple angiogenic checkpoints. This multimodal interference is characteristic of phytochemical mixtures, which exert synergistic effects by targeting several molecular pathways simultaneously. Such broad-spectrum anti-angiogenic activity supports the potential use of these extracts in conditions characterized by pathological angiogenesis, including cancer, diabetic retinopathy, and rheumatoid arthritis.

5. Conclusion

The findings of this study demonstrate that the ethyl acetate fraction of *Pycnanthus angolensis* stem bark exhibits remarkable anticancer potential against MCF-7 human breast cancer cells, as evidenced by strong cytotoxic activity, a high selectivity index relative to normal cells, and potent induction of apoptosis. The fraction was found to exert a comparable apoptotic effect to doxorubicin, a standard chemotherapeutic drug, but with a more favorable selectivity profile, suggesting a potential for reduced systemic toxicity.

Phytochemical analysis revealed the presence of bioactive constituents such as alkaloids, flavonoids, tannins, and phenolic compounds, which have been widely reported to exert anticancer effects through modulation of oxidative stress, interference with cell cycle progression, and activation of apoptotic pathways. Given the growing interest in plant-derived anticancer agents, the present work highlights *P. angolensis* as a promising candidate for further development. Future research should focus on bioassay-guided fractionation to isolate and structurally characterize the active compounds, elucidation of the precise molecular targets and signaling pathways involved, and in vivo evaluation to confirm efficacy and safety. Furthermore, the potential synergistic interactions between *P. angolensis* extracts and existing chemotherapeutics such as doxorubicin merit exploration, as such combinations could lead to enhanced efficacy and reduced drug dosages. The results from this study not only contribute to the growing body of knowledge on the medicinal potential of African ethnomedicinal plants but also provide a scientific basis for the

traditional use of *P. angolensis* in cancer-related ailments. Ultimately, translating these findings into clinically useful therapies will require multidisciplinary collaboration encompassing pharmacognosy, natural products chemistry, pharmacology, molecular biology, and oncology.

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Conflicts of interest

We have none to declare.

Author contributions

CAU: Conceptualization, methodology, data analysis, manuscript draft, and review. **TSM:** Methodology, data analysis, data curation, and review of manuscript. **HBV:** Methodology, visualization, interpretation and editing. **EOI:** Supervision, methodology, software and editing. All authors read and approved the manuscript for submission.

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