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Exploring the Cytotoxic and Antiproliferative Properties of Kalanchoe pinnata Leaf Extracts on Human Breast Cancer (MCF-7) Cells: An Investigation of Phytochemicals and In Vitro Cytotoxicity

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Abstract: ***Kalanchoe pinnata (Lam.) Pers. (generally called the 'air plant' or 'life plant') is a succulent herb documented for use in traditional medicine systems owing to its extensive pharmacological properties. This study assessed the phytochemical components and cytotoxic effects of aqueous and ethanolic leaf extracts of the plant against the human breast adenocarcinoma cell line MCF-7. Fresh leaves were macerated separately in 95% ethanol and distilled water for 48 h, then filtered to establish the extraction yield and loss on drying. Qualitative phytochemical screening was carried out for carbohydrates, glycosides, flavonoids, alkaloids, saponins, triterpenes, phytosterols, tannins, phenolic compounds, and proteins/amino acids. Cytotoxicity was assessed using the MTT assay over a concentration range of 20-100 µg/mL after 24 h of treatment, and cell morphology was examined through acridine orange-ethidium bromide staining. The ethanolic extract showed greater recovery (3.4%) than the aqueous extract (1.2%), along with a broader range of secondary metabolites. Both extracts decreased MCF-7 viability in a concentration-dependent fashion, yet the ethanolic extract was markedly more potent (IC_{50} = 38.18 µg/mL vs. 73.45 µg/mL). AO-EB staining supported these findings, revealing progressive morphological alterations as extract concentrations increased. The current study therefore offers evidence supporting the traditional use of K. pinnata and points to the potential phytoconstituents in its ethanolic leaf extract that could account for its anti-proliferative action against MCF-7 breast cancer cells, justifying further mechanistic and in vivo research.***

Keywords: Kalanchoe pinnata; phytochemical screening; MTT assay; MCF-7 breast cancer cell; AO-EB staining and cytotoxicity

1. Introduction

Cancer ranks among the leading causes of illness and death globally, and breast cancer remains the most frequently occurring malignancy in women. Conventional chemotherapeutic drugs are frequently constrained by dose-limiting toxicities and the emergence of resistance. This has driven renewed interest in investigating plant-derived natural products as sources of new and better-tolerated anticancer agents. Kalanchoe pinnata (Lam.) Pers. (family Crassulaceae), commonly referred to by various names such as the "air plant," "life plant" or "cathedral bells," is a succulent perennial herb that is known to be distributed across tropical and subtropical regions. In several cultures, its leaves are traditionally used to treat wounds, infections, inflammation, and tumours. Its therapeutic reputation is credited to its diverse phytochemical composition, which includes flavonoids, phenolic acids, alkaloids, triterpenoids and bufadienolides, reported to possess anti-inflammatory, antioxidant, antimicrobial, wound healing and antidiabetic activities.

An increasing number of contemporary pharmacological studies have provided evidence supporting several of these traditional claims. Ethanol and aqueous leaf extracts of K. pinnata have been reported to produce selective cytotoxic and pro-apoptotic effects on various human cancer cell lines, such as breast (MCF-7), colon (HT-29) and prostate (PC-3) lines, through reactive oxygen species (ROS) generation, mitochondrial membrane depolarisation and caspase activation. More recently, constituents of K. pinnata such as quercetin and luteolin have been linked to the modulation of epitranscriptomic regulators of breast cancer cell migration.

Because the composition and bioactive potential of plant extracts vary according to the nature and polarity of the solvent used, a systematic extraction and screening procedure is essential before biological evaluation. Therefore, the present study aimed to (i) prepare and characterise aqueous and ethanolic extracts of K. pinnata leaves, (ii) carry out phytochemical screening of the extracts, and (iii) assess the cytotoxic and antiproliferative effects of the extracts on the MCF-7 cell line using MTT assay and AO-EB staining.

2. Materials and Methods

2.1 Plant Material

Fresh leaves of Kalanchoe pinnata were collected and thoroughly cleaned to remove surface dirt and debris. Leaves were chosen based on maturity and overall health to ensure consistency and quality of the extracted phytoconstituents.

2.2 Preparation of Extracts

Two extracts were prepared in parallel by cold maceration. For the ethanolic extract, 100 g of fresh leaves were macerated in 80 mL of 95% ethanol in a clean, sterile glass container. For the aqueous extract, a separate 100 g portion of fresh leaves were macerated in 80 mL of distilled water.

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Both mixtures were macerated for 48 h at room temperature to allow sufficient diffusion of phytoconstituents into the respective solvents. Ethanol was employed as the organic solvent due to its capacity to dissolve a broad range of phytochemicals, including lipophilic terpenoids and phenolics, whereas water was used to preferentially recover hydrophilic constituents such as polysaccharides and flavonoid glycosides.

After maceration, each mixture was filtered through Whatman filter paper (re-filtered if necessary) to separate the liquid extract from residual plant material and particulate matter. The filtrates were used to determine extraction yield and loss on drying, and were retained for phytochemical screening and biological assays.+

2.3 Phytochemical Screening

The aqueous and ethanolic extracts were subjected to standard qualitative phytochemical screening to detect the presence of carbohydrates (Fehling's test), glycosides (Legal test), flavonoids (lead acetate test), alkaloids (Dragendorff test), saponins (foam test), triterpenes (Salkowski test), phytosterols (Liebermann–Burchard test), tannins and phenolic compounds (ferric chloride test) and proteins/amino acids (Millon's and ninhydrin reagents). Each test was performed according to established colorimetric or precipitation-based protocols, and a positive result was recorded as the characteristic colour change, precipitate formation or foam formation described for the reagent.

2.4 Cell Culture

The human breast adenocarcinoma cell line MCF-7 was used in the study. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cryopreserved cells were thawed in a 37°C water bath and transferred to sterile culture flasks containing pre-warmed complete medium. Cultures were maintained in a CO_2 incubator at 37°C with 5% CO_2 and monitored daily by inverted-microscope examination for morphology, adherence, confluence and contamination evidence.

2.5 MTT Cytotoxicity Assay

MCF-7 cells were seeded into 96-well plates at a density of 1.5 x 10^4 cells/well and allowed to attach for 24 h (37°C, 5% CO_2). Stock solutions of the aqueous and ethanolic K. pinnata extracts were prepared in dimethyl sulfoxide (DMSO) and diluted with phosphate-buffered saline (PBS) to final working concentrations of 20, 40, 60, 80 and 100 µg/mL. Diluted extracts were added to the seeded wells and incubated for 24 h at 37°C with 5% CO_2. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (0.5 mg/mL in PBS) was then added (100 µL/well) and the plates were incubated for a further 4 h at 37°C. The medium containing MTT was removed and 100 µL of DMSO was added to solubilise the formazan crystals formed by metabolically viable cells. Absorbance was read at 570 nm on a microplate reader and percentage cell viability and cytotoxicity were calculated relative to untreated controls, and the half-maximal inhibitory concentration (IC_{50}) was derived from the resulting concentration–response curve for each extract.

2.6 Acridine Orange–Ethidium Bromide (AO-EB) Dual Staining

A functional AO-EB stain was made by diluting the stock solution in PBS until the final concentration reached 100 µg/mL. At the 0-hour time point, 10 µL of AO-EB stain was introduced into the wells holding cells that had been treated with the aqueous and ethanolic extracts. The plates were incubated for 24 h (37°C, 5% CO_2), after which the stained cells were viewed under a fluorescence microscope so that morphological changes and viability could be compared between extract-treated and control cells.

3. Results

3.1 Extraction Yield and Loss on Drying

The ethanolic extraction procedure resulted in a notably greater recovery of solid material than aqueous maceration, yielding 3.4% versus 1.2% for the aqueous extract (Table 1). Loss on drying was comparable for the two extracts (1.4% for the ethanolic extract vs. 1.1% for the aqueous extract), suggesting that both preparations had been suitably concentrated prior to downstream testing.

Table 1: Extraction yield and loss on drying for aqueous and ethanolic K. pinnata leaf extracts

Parameter	Aqueous extract	Ethanolic extract
Extraction yield (%)	1.2	3.4
Loss on drying (%)	1.1	1.4

3.2 Phytochemical Screening

Qualitative screening (Table 2) showed that both extracts contained abundant bioactive secondary metabolites. Flavonoids, alkaloids, saponins, phytosterols, tannins, phenolic compounds, and proteins/amino acids were found in both the aqueous and ethanolic extracts. Glycosides were found only in the ethanolic extract, whereas triterpenes appeared only in the aqueous extract, which reflects the differing polarity and solvating capacity of ethanol and water for these classes of compounds.

Table 2: Qualitative phytochemical screening of aqueous and ethanolic K. pinnata leaf extracts

S. No	Constituent	Chemical test	Aqueous extract	Ethanolic extract
1	Carbohydrates	Fehling's test	Present	Present
2	Glycosides	Legal test	Absent	Present
3	Flavonoids	Lead acetate test	Present	Present
4	Alkaloids	Dragendorff test	Present	Present
5	Saponins	Foam test	Present	Present
6	Triterpenes	Salkowski test	Present	Absent
7	Phytosterols	Liebermann–Burchard test	Present	Present
8	Tannins	Ferric chloride test	Present	Present
9	Phenolic compounds	Ferric chloride test	Present	Present
10	Proteins / amino acids	Millon's / ninhydrin reagent	Present	Present

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3.3 Cytotoxic Effects on MCF-7 Cells (MTT Assay)

Across the 20-100 µg/mL range tested, both extracts decreased MCF-7 viability in a concentration-dependent fashion (Tables 3 and 4). At lower concentrations (20-40 µg/mL), the aqueous extract caused mild cytotoxicity, while from 60 µg/mL upwards it caused moderate cytotoxicity; cell viability fell from 79% at 20 µg/mL to 35% at 100 µg/mL, with an IC_{50} of 73.45 µg/mL.

Table 3: Concentration-dependent cytotoxicity of the aqueous K. pinnata extract on MCF-7 cells (24 h treatment, MTT assay)

Concentration (µg/mL)	Cytotoxicity (%)	Cell viability (%)	Cytotoxic response	IC_{50} (µg/mL)
20	21	79	Mild	73.45
40	32	68	Mild	—
60	45	55	Moderate	—
80	51	49	Moderate	—
100	65	35	Moderate	—

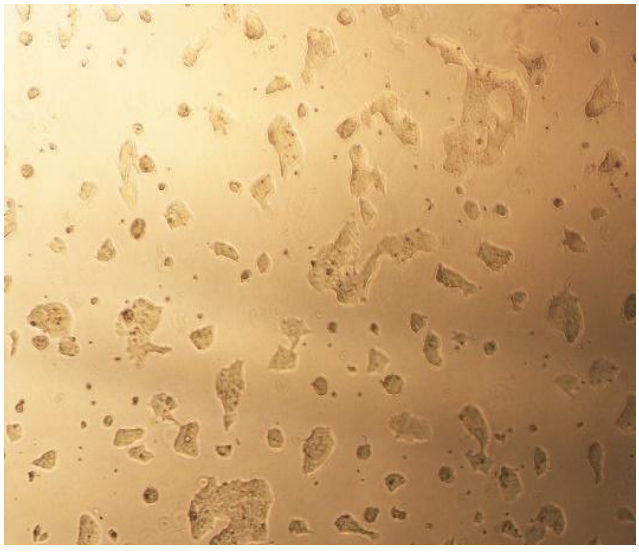
The ethanolic extract proved more potent, causing moderate cytotoxicity starting at the lowest concentration tested (20 µg/mL) and severe cytotoxicity at 80 and 100 µg/mL, where cell viability fell to 24% and 15%, respectively. Its IC_{50} was 38.18 µg/mL, roughly half that of the aqueous extract, which points to markedly stronger antiproliferative activity.

Table 4: Concentration-dependent cytotoxicity of the ethanolic K. pinnata extract on MCF-7 cells (24 h treatment, MTT assay)

Concentration (µg/mL)	Cytotoxicity (%)	Cell viability (%)	Cytotoxic response	IC_{50} (µg/mL)
20	35	65	Moderate	38.18
40	55	45	Moderate	—
60	65	35	Moderate	—
80	76	24	Severe	—
100	85	15	Severe	—

3.4 Cell Morphology and Viability Assessed by Microscopy and AO-EB Staining

Before treatment, phase-contrast microscopy of MCF-7 cultures verified normal adherent, spindle-to-polygonal morphology and the absence of contamination.



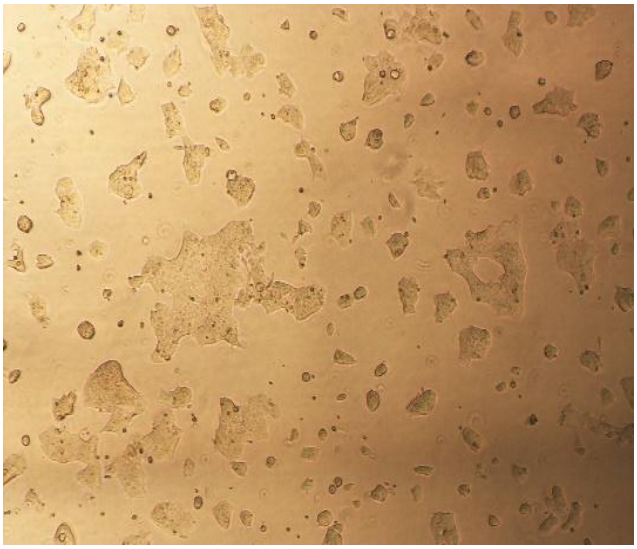


Figure 1: Phase-contrast micrographs representative of untreated MCF-7 cells, demonstrating normal morphology, adherence, and no contamination before extract treatment

After 24-hour treatment and AO-EB staining, cultures exposed to the extract showed concentration-related morphological alterations that matched the MTT viability results, such as changes in cell shape and lower density relative to controls, with the most pronounced effects in wells treated with the ethanolic extract (Figure 2).

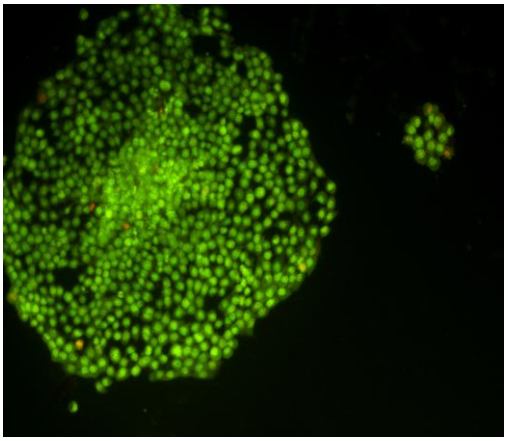


Figure 2: Fluorescence micrographs of MCF-7 cells at 0 hours after AO-EB staining, comparing cells treated with the aqueous and ethanolic extracts

Overall, the microscopy and staining findings align with the concentration- and solvent-dependent cytotoxicity profiles determined by the MTT assay.

4. Discussion

These results show that the aqueous and ethanolic leaf extracts of Kalanchoe pinnata exert concentration-dependent cytotoxic activity against MCF-7 human breast cancer cells, and that the ethanolic extract is roughly twice as potent as the aqueous extract (IC_{50} of 38.18 µg/mL versus 73.45 µg/mL). This discrepancy is probably connected to the phytochemical profile, which showed that the ethanolic extract tested positive for glycosides that were absent from the aqueous extract. Ethanol tends to recover flavonoid aglycones, phenolic acids and triterpenoid-related compounds more effectively than water, and these have

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repeatedly been linked in the literature to the cytotoxic activity of K. pinnata.

These findings agree with independent studies reporting cytotoxicity for K. pinnata. Faundes-Gandolfo et al. (2024) demonstrated that an ethanolic leaf extract of K. pinnata contained abundant phenolic constituents- particularly quercetin and kaempferol- and produced selective, ROS-mediated cytotoxicity toward MCF-7 and other human cancer cell lines while leaving normal colon cells unharmed, pointing to oxidative stress and mitochondrial membrane depolarisation as contributing mechanisms. More recently, Alvizo-Rodríguez et al. (2025) found that an aqueous K. pinnata extract lowered viability in MCF-7 and HCC1937 breast cancer cells and altered the m^{6}A methylation machinery (METTL3/FTO) associated with cancer cell migration- offering a plausible molecular explanation for the biological activity of aqueous preparations, even though their direct cytotoxicity in assays such as the one used here was comparatively weaker. In contrast, Stefanowicz-Hajduk et al. (2020, 2023) observed only weak cytotoxicity for K. pinnata ethanol and water extracts across a panel of cancer cell lines that included MCF-7, suggesting that the level of activity reported for this species can differ substantially depending on plant provenance, extraction conditions and assay design.

The phytochemical screening results reported here- the consistent presence of flavonoids, alkaloids, saponins, phytosterols, tannins and phenolic compounds in both extracts- agree with published phytochemical surveys of K. pinnata, which likewise describe a metabolite profile dominated by flavonoids, alkaloids, triterpenes, glycosides and bufadienolides. A number of these compound classes, especially flavonoids and phenolics, have been documented as having antioxidant and pro-apoptotic properties that might underlie the antiproliferative effects seen in the MTT assay and the morphological changes observed with AO-EB staining.

Certain limitations should be recognised. Cytotoxicity was evaluated at a single 24-hour time point using a colorimetric viability endpoint and qualitative fluorescence staining, with no confirmatory assays such as flow cytometry, caspase activity measurement, or comparison with a normal (non-cancerous) cell line to establish selectivity. The phytochemical screening carried out was qualitative rather than quantitative, and the specific bioactive constituents responsible for the observed cytotoxicity were neither isolated nor identified. Future work should therefore include dose–response studies over longer exposure periods, selectivity indices against normal breast epithelial cells, and bioassay-guided fractionation combined with chromatographic techniques (e.g., HPLC or GC-MS) to pinpoint the specific compounds driving the antiproliferative activity of the ethanolic extract.

5. Conclusion

This study shows that leaf extracts of Kalanchoe pinnata have measurable, concentration-dependent cytotoxic activity against MCF-7 human breast cancer cells, with the ethanolic extract being considerably more potent than the aqueous extract. Phytochemical screening indicates that both extracts are rich in flavonoids, alkaloids, saponins, phytosterols, tannins and phenolic compounds, which are commonly linked to anticancer bioactivity throughout the wider K. pinnata literature. These findings lend experimental support to the traditional medicinal use of K. pinnata and identify its ethanolic leaf extract as a promising candidate for further phytochemical fractionation and mechanistic study as a source of plant-derived antiproliferative agents.

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