RESEARCH PAPER

Evaluation of the Anticancer Potential of Balanites aegyptiaca Leaf on Human Cancer Cell Lines

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ABSTRACT

Background: Globally, malignant tumors remain a primary factor in mortality rates, highlighting an urgent clinical demand for novel, targeted, and biocompatible therapies extracted from botanical origins. *Balanites aegyptiaca* is well-regarded in historical ethnopharmacology for its rich profile of secondary metabolites, particularly steroidal saponins. Nevertheless, empirical validation regarding its therapeutic efficacy against distinct human malignancies remains insufficient, demanding rigorous in vitro exploration.

Objective: The objective of this study was to investigate the in vitro cytotoxic and antiproliferative effects of the methanolic extract of Balanites aegyptiaca leaf on various human cancer cell lines, while also evaluating its selectivity for normal cells.

Methods: The cytotoxic and growth-inhibitory effects were evaluated utilizing MTT and Sulforhodamine B (SRB) assays. Cancer cell lines comprised lung (A549, Hop-62), breast (MCF-7, MDA-MB-231), and cervical (HeLa) cells, whereas normal cell lines consisted of human dermal fibroblasts (HDF), lung fibroblasts (MRC-5), and breast epithelial cells (MCF-10A). Adriamycin (doxorubicin) served as the positive control. Statistical analysis of dose-response data was conducted using ANOVA.

Result: The extract exhibited a dose-dependent decrease in cell viability and proliferation in all cancer cell lines. In the MTT experiment, the IC_{50} values were 39.7 µg/mL for A549 cells, 48.7 µg/mL for MCF-7 cells, and 152.1 µg/mL for HeLa cells. The SRB assay demonstrated growth suppression with GI_{50} values of 49 µg/mL for HeLa cells, 58 µg/mL for Hop-62 cells, and 35 µg/mL for MDA-MB-231 cells. The extract demonstrated selective cytotoxicity, exhibiting significantly reduced toxicity towards normal cell lines (MRC-5, MCF-10A, and HDF). Statistical analysis demonstrated highly significant differences among concentrations and cell types ($p < 0.001$), affirming a pronounced dose-dependent lethal effect.

Conclusion: Balanites aegyptiaca leaf extract has significant and selective anticancer efficacy against lung, breast, and cervical cancer cell lines, suggesting its promise as a natural resource for the formulation of safer anticancer medicines.

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1. INTRODUCTION

On a global scale, oncology management faces severe challenges as neoplasms persist as a monumental threat to public health, claiming millions of lives annually (Sung et al., 2021). Even with substantial technological milestones achieved in early diagnostic screenings and targeted therapeutic interventions, survival indices drop sharply across several demographics. This trend is exacerbated by late-stage clinical presentations, financial barriers to modern healthcare systems, and aggressive tumor relapses (Bray et al., 2018). Among these conditions, carcinomas of the lung, breast, and cervix constitute an overwhelming majority of the documented cases and subsequent fatalities. While these specific cancers display divergent histopathological traits and distinct clinical outcomes, their cumulative pressure on modern healthcare infrastructures is immense. For this reason, identifying innovative pharmacological avenues that maximize tumor regression while minimizing host systemic toxicity is a paramount objective in clinical oncology (Singh et al., 2023).

Current clinical applications of classic antineoplastic therapies face severe restrictions due to narrow therapeutic windows. Standard cytotoxic drugs target rapidly expanding cell populations indiscriminately, meaning that normal, highly regenerative host tissues face structural degradation alongside tumor cells. Consequently, oncological patients routinely face multi-organ

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complications, particularly bone marrow suppression, severe gastrointestinal mucosal damage, and dermatological toxicities. Over prolonged treatment courses, tumors frequently initiate adaptive molecular mechanisms—including drug efflux pump upregulation or mutation of cellular drug binding targets—which diminish baseline drug sensitivity, trigger treatment failure, and accelerate systemic disease progression. Addressing these pharmacological failures requires discovering alternative natural complexes that possess targeted cytotoxic behavior with reduced systemic toxicity to healthy organs (Was et al., 2022).

Currently, systemic cytotoxic chemotherapy constitutes the backbone of standard cancer care protocols. Nonetheless, its therapeutic index is severely narrowed by systemic toxicities and debilitating patient side effects. Broad-spectrum antineoplastic drugs—specifically anthracyclines like doxorubicin—are routinely integrated into regimes combating breast and pulmonary lesions; unfortunately, their utility is fundamentally restricted by cumulative cardiotoxicity and severe bone marrow suppression (Juthani et al., 2024). Because standard cytostatic agents primarily target rapid cellular replication, they struggle to selectively differentiate between hyperproliferative malignant populations and normal somatic tissues. This lack of selectivity induces unintended destruction in highly regenerative sites, including the intestinal mucosa, hematopoietic lineages, and cardiac fibers (J. Sharma, 2011).

Another major challenge in chemotherapy is the development of drug resistance. Cancer cells can develop resistance through various mechanisms, including increased drug efflux, genetic mutations, enhanced DNA repair capacity, and evasion of apoptosis (Holohan et al., 2013). Drug resistance significantly reduces treatment efficacy and contributes to tumor recurrence and disease progression.

In current preclinical oncology screenings, determining a compound's safety index by running parallel evaluations on healthy somatic lineages alongside malignant cells is a vital prerequisite. A structurally optimized therapeutic candidate must selectively disrupt the proliferation networks of cancerous targets while preserving the physiological integrity of adjacent unmutated host tissues. Incorporating healthy cell lines establishes a baseline for therapeutic selectivity and safety profiles prior to considering translational in vivo experiments.

In this research, cellular survival and proliferation kinetics were examined via two fundamentally distinct colorimetric methodologies: the MTT and Sulforhodamine B (SRB) assays. The MTT system determines metabolic health by measuring mitochondrial dehydrogenase functionality in living cells, whereas the SRB technical protocol assesses cumulative cell biomass by staining total cellular protein content. Using these complementary endpoints delivers a thorough, cross-validated assessment of the plant extract's antiproliferative and cytolytic properties.

2. METHODOLOGY

2.1 Collection of Plant Material

The experimental plant i.e. *Balanites aegyptiaca* Leaf were collected from Jaipur, Rajasthan and was authenticated by the Department of Botany, Jaipur, University of Rajasthan (RUBL21951).

2.2 Preparation of Plant Sample

To prepare the plant sample, fresh leaves collected from *Balanites aegyptiaca* were detached from the stems and rinsed exhaustively under running tap water two to three times to eliminate surface debris. The cleaned botanical materials were subsequently spread uniformly and subjected to ambient shade-drying at a regulated room temperature oscillating between 32°C and 37°C. This dehydration process was sustained for a duration of two to three weeks until structural moisture was completely minimized. Following thorough desiccation, the brittle leaf tissues were mechanically reduced to a fine, uniform powder utilizing an industrial laboratory homogenizer. The resulting pulverized matrices were collected and securely preserved to serve as the raw material for all subsequent extraction and experimental protocols.

2.3 Preparation of Plant extract

For the extraction process, finely milled and desiccated foliage of *Balanites aegyptiaca* was loaded into a conical glass percolator and completely immersed in absolute methanol for a 20-hour incubation period at ambient temperature. This exhaustive extraction step was executed for a total of four successive cycles, after which the resulting fractions were harvested and passed through filtration media. The combined filtrates were subsequently concentrated by evaporating the solvent using a rotary evaporator operating under low-pressure conditions at a constant temperature of 50°C. Initial moisture removal was achieved using vacuum desiccators, followed by a final freeze-drying (lyophilization) sequence. The thoroughly dried residue was collected, transferred into wide-mouth glass vials, flushed with an inert nitrogen atmosphere prior to sealing, and preserved at -20°C inside a desiccator.

2.4 MTT Assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

To evaluate the cellular survival profile under the influence of *Balanites aegyptiaca* leaf fractions, an MTT colorimetric framework was carried out. This laboratory principle relies upon the biochemical capacity of functional mitochondrial dehydrogenases within metabolically competent cells to reduce the yellow tetrazolium salt into an insoluble, vivid formazan derivative, which is subsequently monitored via spectrophotometric analysis. Somatic populations were initially distributed into 96-well culture microplates and incubated for 24 hours to promote stable substrate adherence, followed by treatment with a gradient of botanical extract concentrations. After the designated

exposure window, the tetrazolium compound was introduced into each well, and the accumulated crystalline product was thoroughly dissolved prior to determining optical density values. Cellular survival indices were normalized and quantified as a percentage corresponding to the unexposed baseline controls (Alley et al., 1988; Lovitt et al., 2013; Mosmann, 1983).

The proportional rate of cellular survival was established using the equation below:

$$\text{Percentage Survival} = \left(\frac{\text{Optical Density of Treated Assays}}{\text{Optical Density of Control Assays}}\right) \times 100$$

A549 (lung cancer) vs MRC-5 (Normal lung fibroblast cells) and MCF-7 (breast cancer) cell line vs MCF-10A (Normal breast epithelial cells)

For the MCF-7 (breast cancer) and A549 (lung cancer) cell lines, cells were seeded in 96-well plates with a comparatively lower working volume per well. After treatment with different concentrations of the leaf extract, the incubation period was maintained as per experimental requirements. Following treatment, MTT reagent was added and incubated for a variable duration (between 1–4 hours). The resulting formazan crystals were solubilized using a detergent-based solution containing sodium dodecyl sulfate (SDS) in dimethylformamide (DMF).

The experimental setup was implemented to assess the specific cytotoxic impact of Balanites aegyptiaca extract of leaves. Cancer cell lines were compared to their corresponding normal cell lines (MCF-10A for breast and MRC-5 for lung) under the same experimental conditions. Cancerous and healthy cells received treatment with a comparable range of extract levels and were processed concurrently to maintain consistency in experimental conditions. The proportion cell survival obtained for every concentration was similarly analyzed among cancerous cells and normal cell lines to determine the specificity of the extract. A more marked reduction of viability in cancer cells than in normal cells was taken as an indication of particular anticancer activity. This comparison allowed to assess the therapeutic potential of the extract, distinguishing its cytotoxic properties on malignant.

HeLa (Cervical cancer) and HDF (Normal-human dermal fibroblast) cell lines

Cells were plated at defined density in 96-well plates and incubated under controlled conditions for the HeLa (cervical cancer) and HDF (human dermal fibroblast) cell varieties. The cells received treatment with different concentrations of Balanites aegyptiaca leaf extract and a conventional anticancer drug, doxorubicin as positive control after attachment. Following 24 h incubation, MTT reagent was added and cells underwent incubation for an additional 3 h to allow sufficient formation of formazan crystals. The crystals were then dissolved in dimethyl sulfoxide (DMSO) and absorbance was read at 570 nm. The experimental approach also involved compared to a conventional anticancer drug, doxorubicin. Cells were treated with different concentrations of Balanites aegyptiaca leaf extract and also treated with fixed concentration of doxorubicin (1 µg/mL) under similar conditions of experimentation. Negative control was untreated cells and the positive control was doxorubicin treated cells. HeLa and HDF cells were processed in parallel to ensure identical the conditions of the experiment To evaluate the relative cytotoxic efficacy of the extract, the proportion cell viability measured for the plant's standard extract was contrasted with that obtained from doxorubicin.

2.5 SRB Assay (Sulforhodamine B)

HeLa (Cervical cancer), MDA-MB-231 (Breast Cancer), and Hop-62 (Lung cancer) cell lines

To evaluate the cytostatic influence of the extract, the Sulforhodamine B (SRB) colorimetric framework was employed. This analytical method determines cellular proliferation by measuring the total mass of cellular proteins within immobilized cultures. Following an incubation interval with the plant extract, cell layers underwent immobilization, exposure to the SRB fluorophore, and subsequent rinsing cycles to eliminate non-bound dye particles. The protein-trapped dye molecules were then liberated into solution, and optical densities were documented utilizing a microplate reader. The resulting dose-response trajectories were utilized to calculate indices of growth restriction (Skehan et al., 1990; Vichai & Kirtikara, 2006).

Following the administration of the experimental treatments, the cell culture assemblies were incubated under controlled physiological conditions for a duration of 48 hours. To terminate the experimental exposure, 50 µL of chilled 30% (w/v) trichloroacetic acid (TCA) was introduced with care into individual wells, achieving a final analytical concentration of 10% TCA. The cell layers were immobilized *in situ* through a 60-minute incubation maintained at 4°C. After removing the underlying supernatant fractions, the plates underwent five successive washing steps with distilled water to remove lingering culture media, remaining TCA matrix, and detached cellular remnants before being allowed to air dry completely.

The immobilized cellular material was stained by introducing 50 µL of a 0.4% (w/v) SRB formulation dissolved in 1% acetic acid per well, followed by a 20-to-30-minute incubation period at ambient temperature. Repeated washing cycles utilizing 1% acetic acid solution were performed to extract non-specifically bound dye molecules, after which the microplates were dried under ambient air conditions.

The protein-complexed fluorophore was subsequently reconstituted via the addition of a 10 mM unbuffered Tris base solution (adjusted to pH ~10.5). Absorbance metrics were gathered at an analytical wavelength of 540 nm against a reference point of 690 nm using an automated plate reader. Because optical density values correspond

linearly to cumulative cellular protein biomass, these measurements serve as an indicator of cell survival (Skehan et al., 1990). Relative cell growth rates were computed for individual plates against baseline untreated control wells utilizing the operational relationship:

$$\text{Growth Percentage} = \left(\frac{\text{Absorbance}_{\text{treated cells}}}{\text{Absorbance}_{\text{control cells}}}\right) \times 100$$

2.6 Statistical Analysis

All experiments were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation (SD). Statistical evaluation was carried out to determine the significance of differences among treatment groups.

For comparisons involving a single factor (different concentrations within the same cell line), one-way analysis of variance (ANOVA) was applied to assess dose-dependent effects. For experiments involving two factors (concentration and cell type: cancerous vs. normal), two-way ANOVA was used to evaluate the individual effects of each factor as well as their interaction. Statistical significance was considered at $p < 0.05$, and results with $p < 0.001$ were regarded as highly significant. The standard deviation (SD) was calculated using the formula:

$$SD = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

3. RESULTS

3.1 MTT Assay

3.1.1 Normal cell line (MRC-5) Vs. lung cancer cell line (A549)

On the basis of lung cancer cell line (**A549** cell line) viability against normal cell line (MRC-5 cell line)**,** the anticancer activity of *Balanites aegyptiaca* leaf was determined. For this 10-160µg/ml concentration of *Balanites aegyptiaca* leaf was used. At the highest concentration (160µg/mL), cancer cell viability reduced significantly to 27.18%, compared to the control (100%). In contrast, the normal cell line exhibited relatively minor reductions in viability, maintaining 71% viability at the same concentration (Table 1, Figures 1 and 2). The extract IC_{50} value was 39.7µg/ml.

This selective cytotoxicity suggests that *Balanites aegyptiaca* leaf extract exerts stronger inhibitory effects on cancerous cells while sparing normal cells to a considerable extent, indicating its potential as a source of anticancer agents.

Table 1: Cell viability of Normal Cell Line (MRC-5 cell line) and lung cancer cell line (**A549** cell line) treated with *Balanites aegyptiaca* leaf extract

Concentrations (µg/mL)	Cell viability (%) Lung Cancer Cell Line	Cell viability (%) Normal Cell Line
Control	100 ± 0.0306	100 ± 0.0263
10	67.53 ± 0.0567	97 ± 0.0335
20	64.46 ± 0.0885	94 ± 0.0312
40	53.85 ± 0.0263	86 ± 0.0332
80	31.86 ± 0.0317	75 ± 0.0298
160	27.18 ± 0.0339	71 ± 0.0284

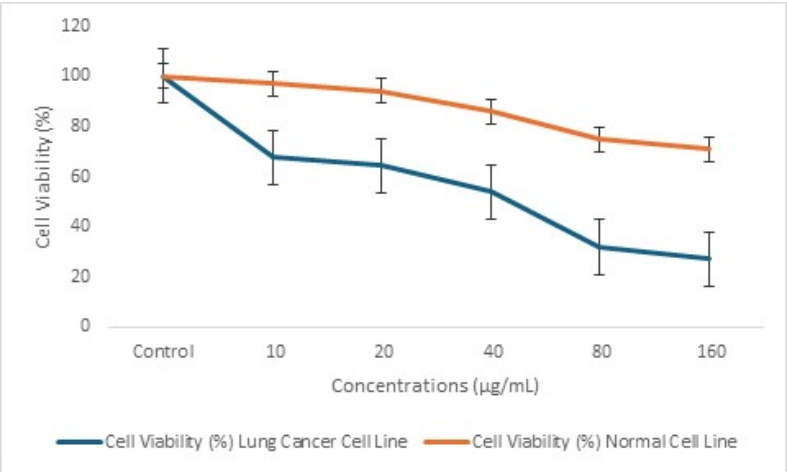


Figure 1: Cell viability of Normal Cell Line (MRC-5 cell line) and lung cancer cell line (**A549** cell line) treated with *Balanites aegyptiaca* leaf extract

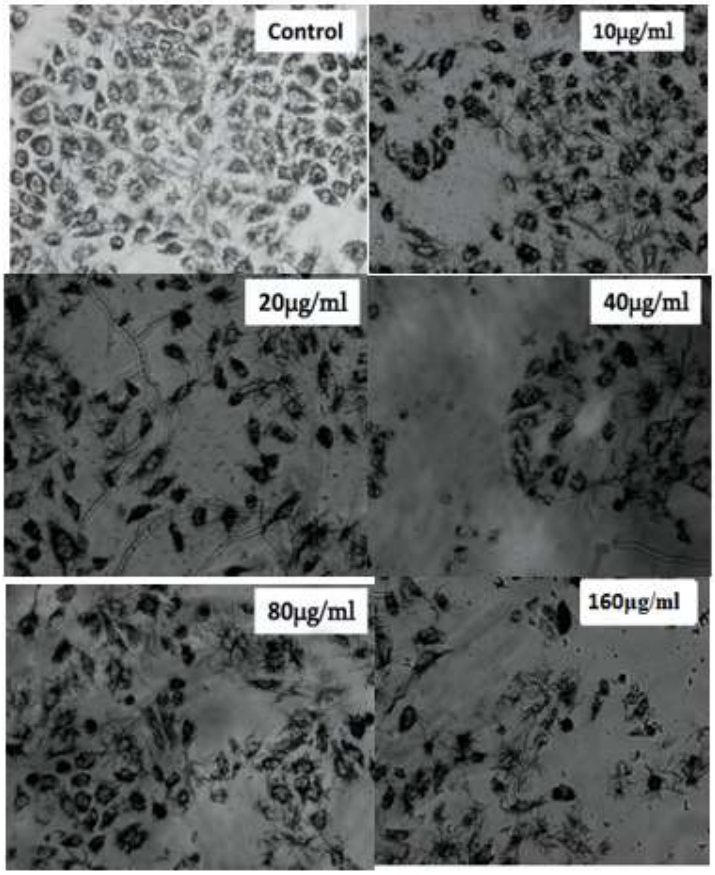


Figure 2: Cell viability of lung cancer cell line against *Balanites aegyptiaca* leaf extract

Statistical Analysis

Cell viability was measured using a two-way ANOVA to determine the impact of standard concentration and cell type. The analysis showed an statistically significant impact of standard concentration on cell viability ($F(5,24) = 1262.63$, $p < 0.001$) demonstrating a clear dose-specific cytotoxic effect of the methanolic extract of Balanites aegyptiaca. There was additionally an important impact of cell line ($F(1,24) = 4598.08$, $p < 0.001$), indicating differences in response among cancerous and normal cells. Moreover, an important connection among concentration and the cell line was found [$F(5,24) = 220.55$, $p < 0.001$], suggesting that the cytotoxic effect of the extract depended on both the concentration of the extract and the type of cell. The extract showed more inhibitory effect on A549 lung cancer cells than on MRC-5 normal lung fibroblast cells, assisting the specific anticancer activity of the standard extract (Table 2).

Table 2: Two-way ANOVA Showing the Effect of Concentration and Cell Line on Cell Viability

Source	df	F-value	p-value
Concentration	5	1262.63	<0.001
Cell Line	1	4598.08	<0.001
Concentration × Cell Line	5	220.55	<0.001
Error	24	—	—

3.1.2 Normal Cell Line (MCF- 10A) Vs. Breast cancer cell line (MCF-7)

In the breast cancer cell line (MCF-7), treatment with 10 µg/ml resulted in a slight reduction in viability to 92.08%, while a significant decrease to 46.48% was observed at 160µg/ml—representing a reduction of approximately 53.5% compared to the control. Similarly, the normal cell line showed a relatively mild decline in viability, from 100% in the control to 70% at the highest tested concentration (160µg/ml), indicating a 30% reduction (Table 3, Figures 3 and 4). The extract IC_{50} value was 48.7µg/ml.

Table 3: Cell viability (%) of Breast Cancer Cell Line and Normal Cell Line against *Balanites aegyptiaca* leaf extract

Tested concentrations (µg/ml)	Cell viability (%) Breast Cancer Cell Line (MCF-7)	Cell viability (%) Normal Cell Line (MCF- 10A)
Control	100 ± 3.97	100 ± 0.026
10	92.082 ± 2.84	98 ± 0.033
20	86.336 ± 3.32	95 ± 0.031
40	79.113 ± 1.53	83 ± 0.033
80	66.889 ± 4.40	74 ± 0.029
160	46.482 ± 2.69	70 ± 0.028

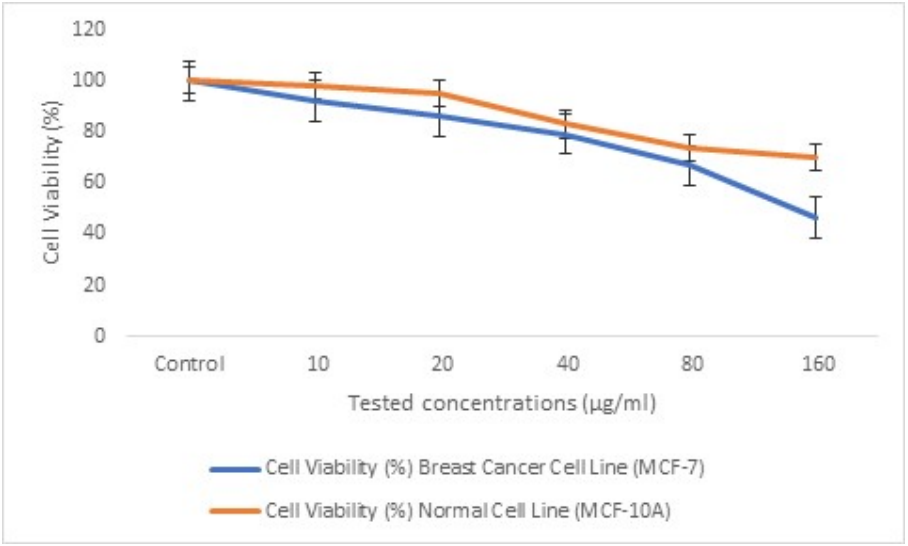


Figure 3: Cell viability of breast cancer cell line (MCF-7) and Normal Cell line (MCF-10A) treated with *Balanites aegyptiaca* leaf extract

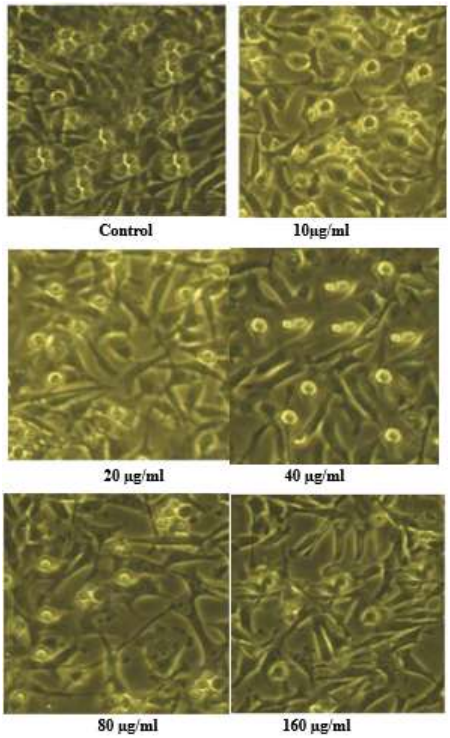


Figure 4: Cell viability of Breast cancer cell line against *Balanites aegyptiaca* leaf extract

Statistical Analysis

The effect of concentration of extract and type of cell-on-cell survival in breast cancer (MCF-7) and healthy breast epithelial (MCF-10A) cell lines was evaluated by two-way ANOVA. The analysis revealed that the impact of the concentration on the cell's survival was statistically noteworthy ($F(5,24) = 285.82$, $p < 0.001$). This result indicates that there is a concentration-related decrease in the cellular viability after treatment with the methanolic extract of Balanites aegyptiaca. The cell line also had a significant effect ($F(1,24) = 113.38$, $p < 0.001$) indicating that malignant and healthy cells responded differently to the chemotherapy. Moreover, the interaction among concentration and cell line was significant ($F(5,24) = 18.46$, $p < 0.001$), suggesting that the cytotoxic response of the extract was dependent on both the concentration and the cell type.

The extract showed a higher detrimental effect on MCF-7 breast cancer cells in comparison with MCF-10A normal breast epithelial cells, supporting its selective anticancer potential and comparatively lower toxicity towards normal cells (Table 4).

Table 4: Two-way ANOVA Showing the Effect of Concentration and Cell Line on Breast Cell Viability

Source	df	F-value	p-value
Concentration	5	285.82	<0.001
Cell Line	1	113.38	<0.001
Concentration × Cell Line	5	18.46	<0.001
Error	24	—	—

3.1.3 *Balanites aegyptiaca* leaf extract concentration Vs. Human Cervical Adenocarcinoma cell line (HeLa) - (MTT Assay)

The cytotoxic activity of Balanites aegyptiaca leaf extract was determined against HeLa cell line by MTT assay at different concentrations of 31.25 - 500 µg/mL (Table 5 and Figures 6 and 7). The viability of untreated control cells was 100 ± 0.03%, and the viability of cells treated with standard anticancer drug doxorubicin (1 µg/mL) was 56.47 ± 1.35%. The concentrate showed a clear concentration-related decrease of survival in HeLa cells. The cell viability at 31.25 µg/mL was 90.08 ± 0.60%, and it gradually decreased to 77.98 ± 1.25% at 62.5 µg/mL. At 125 µg/mL, a significant reduction was observed (57.22 ± 0.85%), which was comparable to the induced cytotoxic effect of doxorubicin. At further increase in concentration, there was significant suppression of cell proliferation and cell survival decreased to 36.88 ± 0.75% at 250 µg/mL and minimum of 15.32 ± 0.19% at 500 µg/mL. The extract showed moderate cytotoxic activity with IC50 value of 152.1 µg/mL.

Table 5: Effect of *Balanites aegyptiaca* leaf extract on HeLa cell line

Concentration (µg/mL)	% Cell Viability of HeLa Cells
Control	100 ± 0.03
Doxorubicin (1 µg/mL)	56.47 ± 1.35
31.25	90.08 ± 0.60
62.5	77.98 ± 1.25
125	57.22 ± 0.85
250	36.88 ± 0.75
500	15.32 ± 0.19

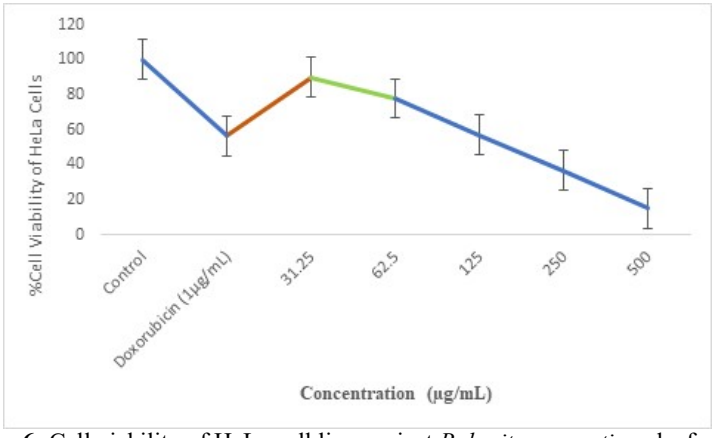


Figure 6: Cell viability of HeLa cell line against *Balanites aegyptiaca* leaf extract

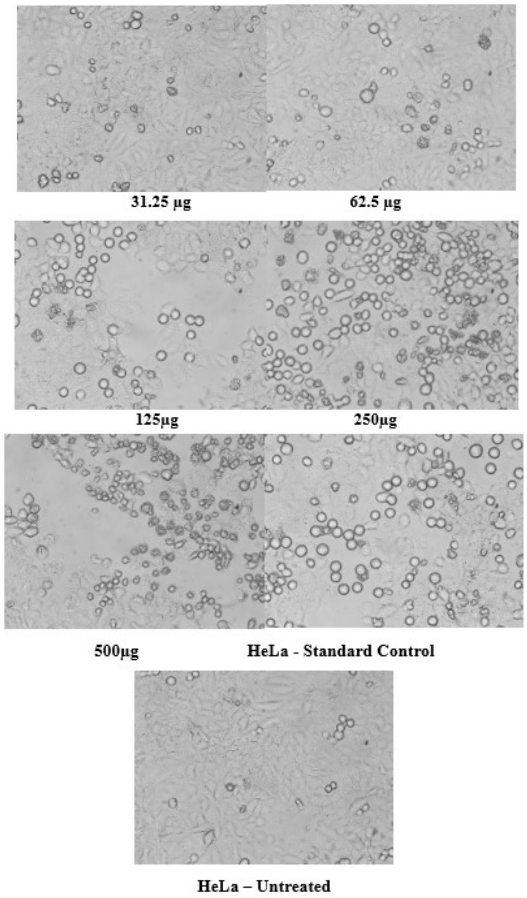


Figure 7: Cell viability of HeLa cell line against different concentrations of *Balanites aegyptiaca* leaf extract

Statistical Analysis

One-way ANOVA showed statistically important variation in the cell survival of HeLa cells treated with varying concentrations of the methanolic extract of Balanites aegyptiaca tested ($F(6,14) = 3696.39$, $p < 0.001$). The findings verified the substantial concentration-specific cytotoxic impact of the standard extract on cervical cancer cells. The cell viability decreased progressively with rising concentrations of extract suggesting more antiproliferative effects at higher concentrations (Table 6). The extract showed moderate cytotoxicity against HeLa cells, but the IC_{50} value observed indicated relatively lower tolerance of HeLa cells compared to A549 and MCF-7 cell lines. These differential responses may be related to differences in cellular metabolism, apoptotic signaling systems and intrinsic immunity properties of cervical cancer cells.

Table 6: One-way ANOVA for % Cell Viability of HeLa Cells (MTT Assay)

Source of Variation	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	16053.94	6	2675.66	3696.39	<0.001
Within Groups	10.13	14	0.72	—	—
Total	16064.07	20	—	—	—

3.1.4 Balanites *aegyptiaca* leaf concentration Vs. Human dermal fibroblast cell line

The cytotoxicity of Balanites aegyptiaca extracts of leaves was determined on the standard Human Dermal Fibroblast (HDF) cell line by the standard MTT assay in the concentration range of 31.25 to 500 µg/mL (Table 7; Figures 8 and 9). Untreated control cells showed 100 ± 0.02 % viability, whereas standard drug doxorubicin (1 µg/mL) reduced the viability to 72.86 ± 1.35 %. The therapy with the plant extract showed a steady decline in the cell survival with the increase of the dosage, but the decrease was relatively moderate. At 31.25 µg/mL the viability was 97.30 ± 0.60%, followed by 95.40 ± 1.25% at 62.5 µg/mL and 93.39 ± 0.85% at 125 µg/mL. The decrease was more prominent at higher concentrations, with viability of 79.41 ± 0.75% at 250 µg/mL and 69.21 ± 0.19% at 500 µg/mL. IC50 values were not determinable (NA) under the study's parameters tested. However, the extract was moderately toxic to the tested cell line. Although a dose dependent reduction in viability was observed, the cytotoxic effect on HDF cells was significantly lower than that on cancer cell lines.

Table 7: Effect of *Balanites aegyptiaca* leaf extract on HDF cell line

Concentration (µg/mL)	% Cell Viability of HDF Cells
Control	100 ± 0.02
Doxorubicin (1 µg/mL)	72.86 ± 1.35
31.25	97.30 ± 0.60
62.5	95.40 ± 1.25
125	93.39 ± 0.85
250	79.41 ± 0.75
500	69.21 ± 0.19

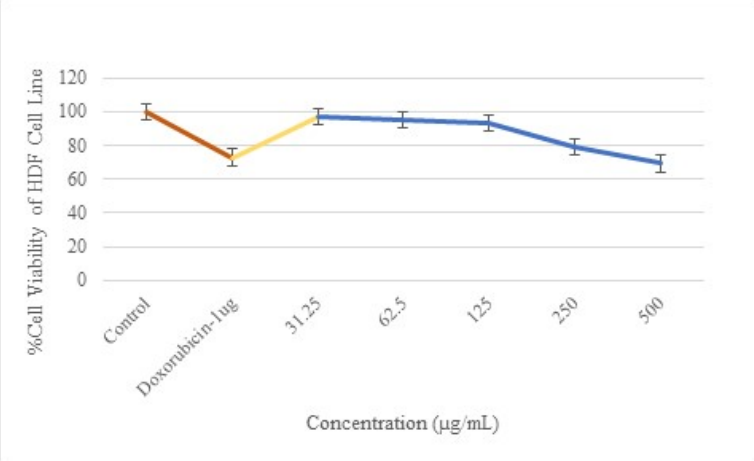


Figure 8: Cell viability of HDF Cell line against *Balanites aegyptiaca* leaf extract

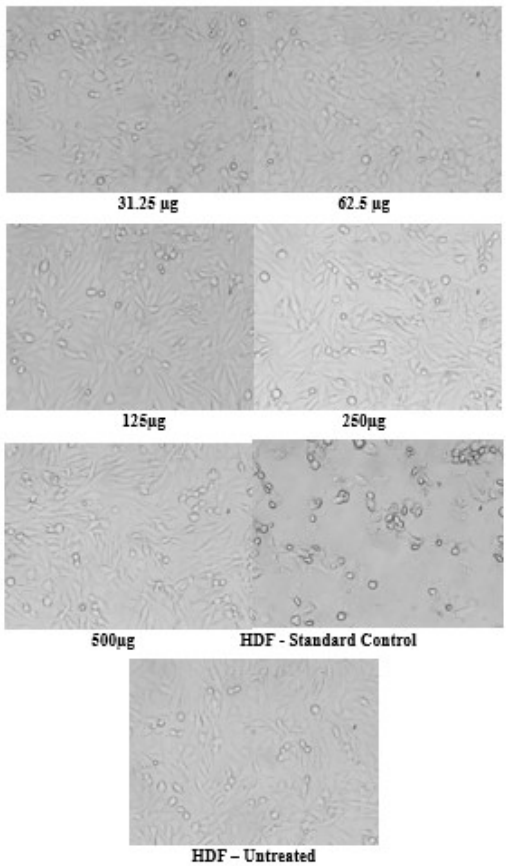


Figure 9: Cell viability of HDF cell line against different concentrations of *Balanites aegyptiaca* leaf extract

Statistical Analysis

One-way ANOVA showed that there was an important distinction in the cell's survival rate between the various concentrations of methanolic extract of Balanites aegyptiaca evaluated against Human Dermal Fibroblast (HDF) cells ($F(6,14) = 663.33$, $p < 0.001$). The results showed that the viability of HDF cells was gradually decreased with increasing concentrations of the extract. The level of cytotoxicity observed in HDF cells was, however, much lower than that observed in the cancer cell lines tested. A good number of normal cells were still viable even at higher concentrations indicating the comparatively less toxicity of the extract towards the normal human cells. This differential response supports the selective anticancer potential of the methanolic extract and suggests its possible therapeutic advantage in minimizing damage to normal tissues during anticancer treatment (Table 8). The relatively lower sensitivity of HDF cells may be related to differences in cellular metabolism, membrane stability, antioxidant defence mechanisms and lower susceptibility to apoptosis induction compared to malignant cells.

Table 8: One-Way ANOVA for % Cell Viability of HDF Cells

Source of Variation	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	2880.67	6	480.11	663.33	<0.001
Within Groups	10.13	14	0.72	—	—
Total	2890.80	20	—	—	—

3.2 SRB Assay

3.2.1 *Balanites aegyptiaca* leaf extract concentration Vs. HeLa Cervical Cancer Cell Line (SRB Assay)

The antiproliferative efficacy of Balanites aegyptiaca extract of leaves versus the HeLa cervical cancer cell line was evaluated using the SRB assay at concentrations of 10, 20, 40 and 80 µg/mL (Table 9; Figures 10 and 11). At lower concentrations (10 and 20 µg/mL), the percentage control growth was 107.0 ± 2.83% and 104.0 ± 2.40%, respectively, indicating a minimum inhibitory effect and a minor cell proliferation relative to the untreated control. Cell growth was significantly decreased at higher concentrations. The percentage control growth was significantly ($p<0.05$) decreased to 48.7±13.70% at 40 µg/mL that is almost 50% inhibition of cell proliferation. The 80 µg/mL concentration showed a significant cytotoxic effect, with the control growth dropping drastically to 9.6 ± 4.60%, suggesting strong inhibition of the HeLa cell proliferation. The leaf extract of Balanites aegyptiaca had a GI50 value of 49 µg/mL on the HeLa cell line with TGI and LC50 values >80 µg/mL indicating no complete growth inhibition and 50% cell death within the tested concentration range.

Table 9: Effect of *Balanites aegyptiaca* leaf extract on HeLa cell line

Concentration (µg/mL)	% Control Growth of HeLa Cell Line
10	107.0 ± 2.83
20	104.0 ± 2.40
40	48.7 ± 13.70
80	9.6 ± 4.60

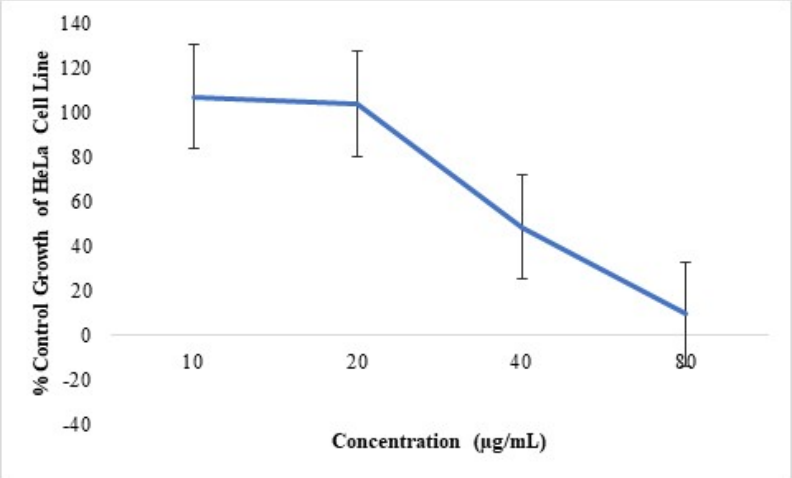
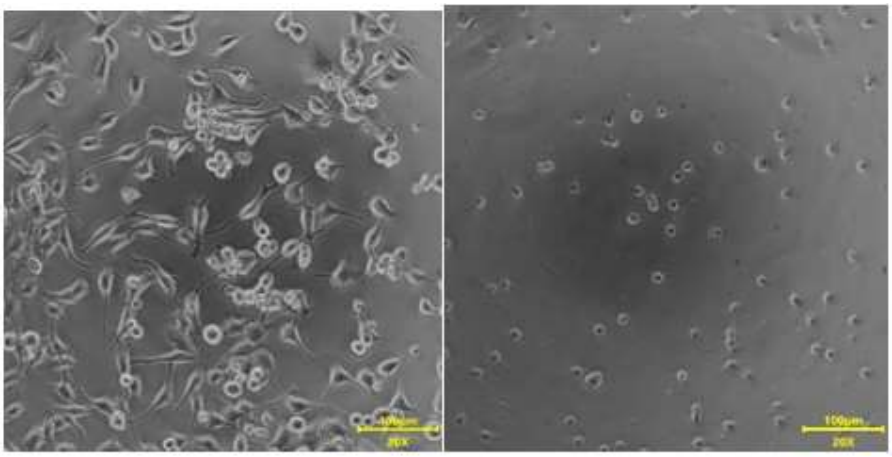


Figure 10: % Control Growth of HeLa Cell Line against *Balanites aegyptiaca* leaf extract



HeLa - Control **HeLa – Leaf Extract of *Balanites aegyptiaca***

Figure 11: Effect of *Balanites aegyptiaca* leaf extract on HeLa Cell Line

Statistical Analysis

To determine the impact of varying extract concentrations on HeLa cervix carcinoma growth, a one-way analysis of variance (ANOVA) was executed, revealing highly meaningful variances across treatment cohorts ($F(3,8) = 118.56, p < 0.001$). Data obtained via the SRB colorimetric framework firmly supported a dose-dependent restriction of cellular proliferation. While minimal growth inhibition was noted at the lowest tested dilution, higher doses triggered an extreme reduction in total biomass. This severe arrest of proliferation at 40 and 80 $\mu\text{g/mL}$ implies that the underlying phytochemicals severely compromise cellular replication and viability pathways within HeLa cells. This statistically backed decline highlights the broad antineoplastic potential of the *Balanites aegyptiaca* methanolic profile.

At lower concentrations the extract exhibited a slight inhibition of proliferation of HeLa cells, however, at higher concentrations, the percentage control growth of HeLa cells was significantly reduced indicating better cytotoxic and growth-inhibitory activity. Marked reduction of cell growth at 40 µg/mL and 80 µg/mL indicates that the extract disrupts the cellular mechanism of proliferation and survival in HeLa cells.

The decrease in the cell growth in a statistically significant manner further confirms the anticancer potential of methanolic extract of Balanites aegyptiaca. The observed antiproliferative activity might be due to the presence of bioactive phytoconstituents such as steroidal saponins, flavonoids and phenolic compounds which have been reported to induce apoptosis, alter mitochondrial function and suppress cancer cell proliferation via oxidative stress mediated mechanisms (Table 10).

Table 10: One-Way ANOVA for % Control Growth of HeLa Cells (SRB Assay)

Source of Variation	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	19794.68	3	6598.23	118.56	<0.001
Within Groups	445.24	8	55.66	—	—
Total	20239.92	11	—	—	—

3.2.2 *Balanites aegyptiaca* leaf extract concentration Vs. Human Lung Cancer Hop-62 Cell Line

The antiproliferative activity of *Balanites aegyptiaca* leaf extract against the human lung cancer Hop-62 cell line was evaluated using the SRB assay at concentrations of 10, 20, 40, and 80 µg/mL (Table 11; Figures 12 and 13).

At lower concentrations, the extract exhibited moderate inhibitory effects. The percentage control growth was 83.2 ± 6.40% at 10 µg/mL and 86.5 ± 3.20% at 20 µg/mL, indicating slight suppression of cell proliferation compared to untreated control cells. At 40 µg/mL, control growth was 78.2 ± 14.00%, suggesting continued but moderate inhibition.

A substantial reduction in cell growth was observed at the highest concentration tested (80 µg/mL), where percentage control growth declined markedly to 25.6 ± 3.10%. This indicates strong inhibition of Hop-62 cell proliferation at higher extract concentrations. For the Hop-62 human lung cancer cell line, the growth inhibition parameters were determined from the dose–response curve. The GI_{50} value for the leaf extract of *Balanites aegyptiaca* was found to be 58 µg/mL. However, the TGI and LC_{50} values were greater than 80 µg/mL, indicating that complete growth inhibition and 50% cell lethality were not achieved within the tested concentration range.

Table 11: Effect of *Balanites aegyptiaca* leaf extract on Hop-62 Cell Line

Concentration (µg/mL)	% Control Growth of Hop-62 Cell Line
10	83.2 ± 6.40
20	86.5 ± 3.20
40	78.2 ± 14.00
80	25.6 ± 3.10

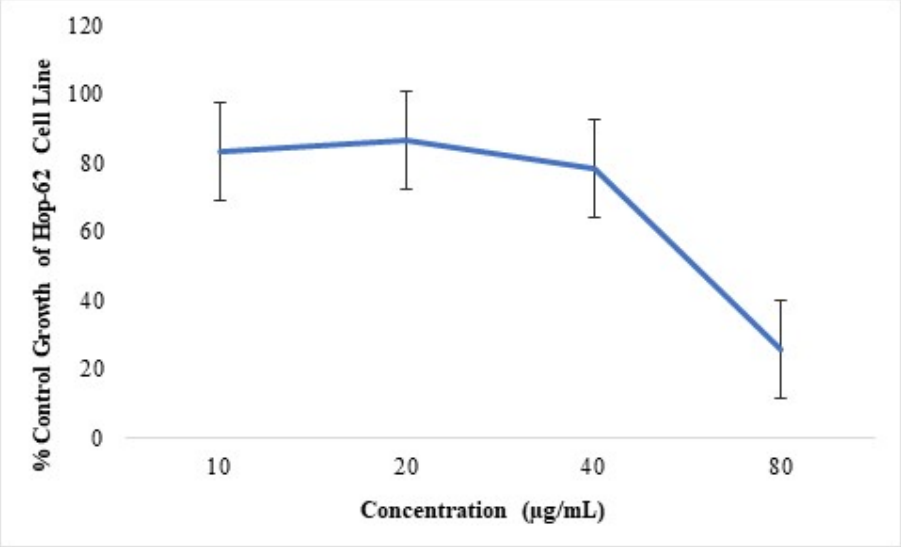


Figure 12: % Control Growth of Hop-62 Cell Line against *Balanites aegyptiaca* leaf extract

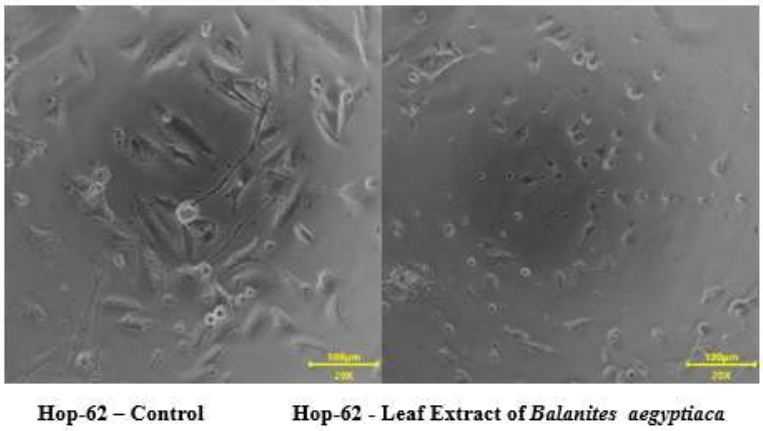


Figure 13: Effect of *Balanites aegyptiaca* leaf extract on Hop-62 Cell Line

Statistical Analysis

The percentage control growth of Hop-62 lung cancer cells treated with different concentrations of methanolic extract of Balanites aegyptiaca showed a significant difference by one-way ANOVA ($F(3,8) = 38.54$, $p < 0.001$). The results confirmed the significant concentration-dependent inhibitory effect of the extract on the proliferation of Hop-62 cells.

The extract showed moderate growth inhibition at lower concentrations while the highest concentration tested (80 µg/mL) showed significant reduction in percentage control growth. The decreasing proliferation of cells indicates that the higher concentrations of the extract are more cytotoxic and antiproliferative against lung cancer cells.

This inhibitory effect may be due to the presence of biologically active phytoconstituents such as steroidal saponins, flavonoids, alkaloids and phenolic compounds in Balanites aegyptiaca. These compounds have been reported to interfere with survival pathways of cancer cells, induce oxidative stress, disrupt the integrity of the mitochondrial membrane and promote apoptosis in malignant cells.

Moreover, the considerably lower GI_{50} value obtained for Hop-62 cells indicates that lung cancer cells are relatively sensitive to the extract. Statistically significant inhibition of cell growth supports the possible use of the methanolic extract of Balanites aegyptiaca as a possible natural anticancer agent for treatment of lung cancer (Table 12).

Table 12: One-Way ANOVA for % Control Growth of Hop-62 Cells

Source of Variation	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	7423.58	3	2474.53	38.54	<0.001
Within Groups	513.62	8	64.20	—	—
Total	7937.20	11	—	—	—

3.2.3 *Balanites aegyptiaca* leaf extract concentration Vs. Human Breast Cancer MDA-MB-231 Cell Line

The cytotoxic and antiproliferative effect of Balanites aegyptiaca leaf extract was tested on the human breast cancer cell line MDA-MB-231 by SRB assay at concentration 10, 20, 40 and 80 µg/mL (Table 13; Figures 14 and 15).

At 10 µg/mL, the percentage control growth was 92.0 ± 2.80% indicating a minimum inhibition of cell proliferation. At 20 µg/mL a moderate reduction in growth was observed with control growth decreasing to 78.3 ± 7.60%. Control growth was reduced dramatically to 35.2 ± 9.60% indicating significant inhibition of cell proliferation, thus revealing a prominent inhibitory activity at 40 µg/mL.

At the highest concentration tested (80 µg/mL), the percentage control growth was −24.6 ± 9.50%. The negative growth value was calculated not only to determine the total inhibition of cell proliferation, but also to determine the reduction in cell number below the initial cell population, which indicates a strong cytotoxic activity and possible induction of cell death. The GI50 value of Balanites aegyptiaca leaf extract was found to be 35 µg/mL against MDA-MB-231 human breast cancer cell line and TGI value was 64 µg/mL. The LC50 value was >80 µg/mL indicating that 50% cell lethality was not achieved in the tested concentration range.

Table 13: Effect of *Balanites aegyptiaca* leaf extract on MDA-MB-231 Cell Line

Concentration (µg/mL)	% Control Growth of MDA-MB-231 Cell Line
10	92.0 ± 2.80
20	78.3 ± 7.60
40	35.2 ± 9.60
80	-24.6 ± 9.50

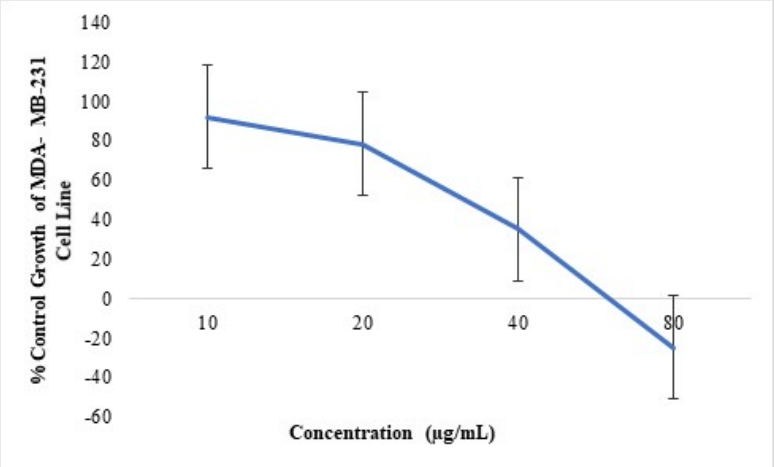


Figure 14: % Control Growth of MDA-MB-231 Cell Line against *Balanites aegyptiaca* leaf extract

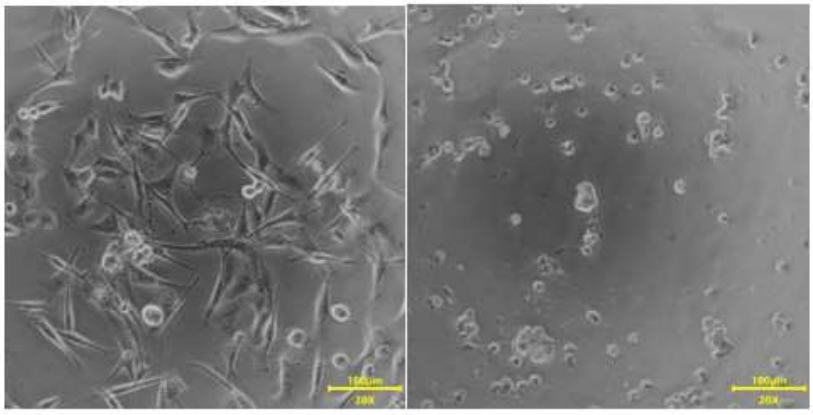


Figure 15: Effect of *Balanites aegyptiaca* leaf extract on MDA-MB-231 Cell Line

Statistical Analysis

One-way ANOVA showed that there was a statistically significant difference in the percentage control growth of MDA-MB-231 breast cancer cells treated with different concentrations of the methanolic extract of Balanites aegyptiaca ($F(3,8) = 133.19$, $p < 0.001$). The results confirmed that the extract exerted a strong concentration-dependent antiproliferative and cytotoxic effect on the highly aggressive breast cancer cell line MDA-MB-231.

The percentage control growth gradually decreased with the increase in the concentration of extract. The extract showed moderate inhibition of cell proliferation at lower concentrations. There was significant inhibition of growth at 40 µg/mL. Interestingly, the negative percentage growth at 80 µg/mL was indicative of complete inhibition of proliferation, as well as a decrease in the number of viable cells below the initial cell number, which points to significant cytotoxicity and induction of cell death.

The strong inhibitory response observed in MDA-MB-231 cells may be related to the aggressive and metabolically active characteristics of triple-negative breast cancer cells, which are typically more vulnerable to oxidative stress-induced damage and apoptosis triggered by phytochemicals. Thus, the observed cytotoxic activity can be attributed to the presence of bioactive constituents such as steroidal saponins, flavonoids, phenolic compounds and terpenoids present in methanolic extract of Balanites aegyptiaca. These phytoconstituents have been earlier reported to interfere with cancer cell proliferation by multiple mechanisms including mitochondrial dysfunction, reactive oxygen species generation, activation of apoptotic pathways and inhibition of survival signalling pathways. The lower GI 50 value obtained for MDA-MB-231 cells also supports the high anticancer potential of the extract against breast cancer cells (Table 14).

Table 14: One-Way ANOVA for % Control Growth of MDA-MB-231 Cells (SRB Assay)

Source of Variation	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	24773.66	3	8257.89	133.19	<0.001
Within Groups	496.02	8	62.00	—	—
Total	25269.68	11	—	—	—

3.2.4 Adriamycin (ADR) concentration Vs. HeLa Cervical Cancer Cell Line (SRB Assay)

The cytotoxic effect of Adriamycin (ADR) on HeLa cervical cancer cell line was determined by SRB assay for the concentrations of 10, 20, 40 and 80 µg/mL (Table 15; Figures 16 and 17).

ADR showed significant cytotoxic activity at all concentrations tested with negative percentage control growth values. The percentage control growth at 10 µg/ml was −47.0 ± 8.00% indicating considerable inhibition of cell proliferation. The inhibitory effect was further increased at 20 µg/mL (−60.5 ± 2.70%) and reached a maximum at 40 µg/mL (−64.8 ± 6.90%) indicating pronounced cytotoxicity and reduction in cell number below the initial population.

% control growth at 80 µg/mL was -40.2 ± 9.40 %. Although still highly cytotoxic, the effect was less pronounced in comparison to 40 µg/mL. In the HeLa cell line, the positive control Adriamycin (ADR) showed potent cytotoxic activity with GI 50, TGI and LC 50 values all <10 µg/mL, which indicates significant growth inhibition and cell killing at low concentrations.

Table 15: Effect of Adriamycin (ADR) on HeLa Cell Line

Concentration (µg/mL)	% Control Growth of HeLa Cells
10	-47.0 ± 8.00
20	-60.5 ± 2.70
40	-64.8 ± 6.90
80	-40.2 ± 9.40

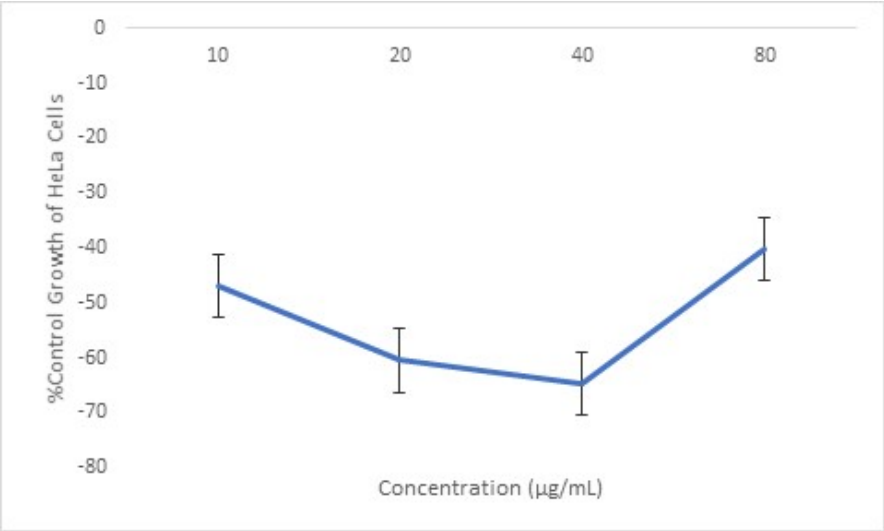
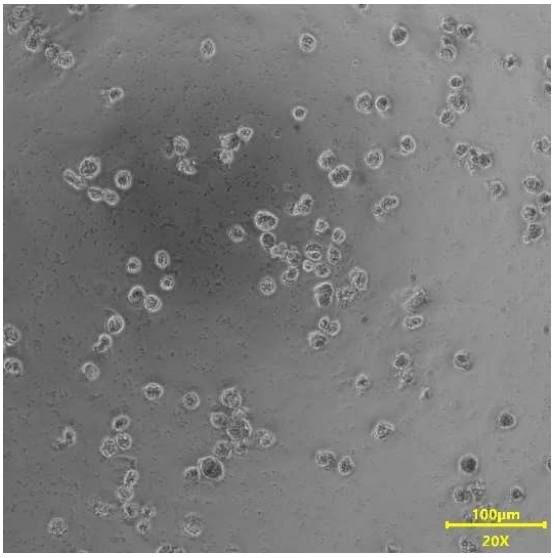


Figure 16: % Control Growth of HeLa Cell Line against Adriamycin (ADR) concentration



HeLa- ADR

Figure 17: Effect of Adriamycin (ADR) on HeLa Cell Line

Statistical Analysis

Table 16: One-Way ANOVA for ADR on HeLa Cell Line (SRB Assay)

Source	SS	df	MS	F	p-value
Between Groups	1185.80	3	395.27	7.62	0.01
Within Groups	414.52	8	51.81	—	—
Total	1600.32	11	—	—	—

One-way ANOVA showed that Adriamycin (ADR) treatment caused a statistically significant difference in % control growth of HeLa cells ($F(3,8) = 7.628$, $p = 0.010$) ($p = 0.010$ (< 0.05), indicating that varying concentrations of ADR significantly affect cell growth and exhibit a concentration-dependent cytotoxic effect on HeLa cells (Table 16).

3.2.5 Adriamycin (ADR) concentration Vs. Human Lung Cancer Hop-62 Cell Line

The cytotoxic effect of Adriamycin (ADR) on the human lung cancer Hop-62 cell line was evaluated using the SRB assay at concentrations of 10, 20, 40, and 80 µg/mL (Table 17; Figures 18 and 19).

At 10 µg/mL, ADR markedly suppressed cell growth, with percentage control growth reduced to 8.4 ± 6.70%, indicating strong inhibition of proliferation. However, at 20 µg/mL and 40 µg/mL, percentage control growth increased to 32.0 ± 3.50% and 53.2 ± 5.50%, respectively, suggesting comparatively reduced cytotoxicity at these intermediate concentrations. At 80 µg/mL, control growth was 43.0 ± 3.60%, still reflecting significant inhibition compared to untreated control cells. For the Hop-62 human lung cancer cell line, the positive control Adriamycin (ADR) showed significant growth inhibitory activity with a GI_{50} value <10 µg/mL, while the TGI and LC_{50} values were non-evaluable (NE) under the tested conditions. ADR demonstrated substantial growth inhibitory effects on Hop-62 cells across all tested concentrations, although the response was not strictly linear with increasing dose.

Table 17: Effect of Adriamycin (ADR) on Hop-62 Cell Line

Concentration (µg/mL)	% Control Growth of Hop-62 Cells
10	8.4 ± 6.70
20	32.0 ± 3.50
40	53.2 ± 5.50
80	43.0 ± 3.60

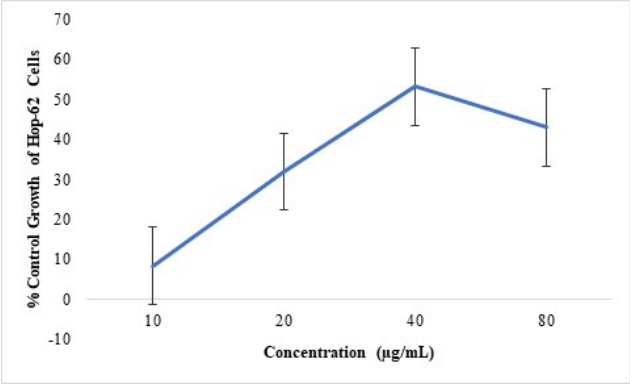
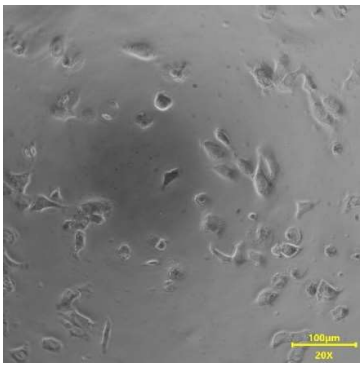


Figure 18: % Control Growth of Hop-62 Cell Line against Adriamycin (ADR) concentration



Hop-62 – ADR

Figure 19: Effect of Adriamycin (ADR) on Hop-62 Cell Line

Statistical Analysis

Table 18: One-way ANOVA for % Control Growth of Hop-62 Cells treated with ADR

Source of Variation	Sum of Squares	df	Mean Square	F value	p-value
Between Groups	3326.73	3	1108.91	44.20	< 0.001
Within Groups	200.70	8	25.09	—	—
Total	3527.43	11	—	—	—

One-way ANOVA revealed a highly significant effect of Adriamycin (ADR) concentration on the % control growth of Hop-62 cells ($F(3,8) = 44.202$, $p < 0.001$), indicating that ADR significantly inhibits the proliferation of Hop-62 cells, with increasing concentrations leading to greater cytotoxic effects (Table 18).

3.2.6 Adriamycin (ADR) concentration Vs. Human Breast Cancer MDA-MB-231 Cell Line

The cytotoxic activity of Adriamycin (ADR) against the human breast cancer MDA-MB-231 cell line was assessed using the SRB assay at concentrations of 10, 20, 40, and 80 µg/mL (Table 19; Figures 20 and 21).

ADR exhibited pronounced cytotoxic effects at all tested concentrations, as reflected by negative percentage control growth values. At 10 µg/mL, the percentage control growth was −34.4 ± 1.80%, indicating substantial inhibition of cell proliferation and reduction in viable cell number below the initial cell population. A similar level of inhibition was observed at 20 µg/mL (−32.9 ± 3.60%).

A more marked cytotoxic effect was evident at higher concentrations. At 40 µg/mL, control growth declined to −53.5 ± 10.40%, and the strongest inhibitory effect was observed at 80 µg/mL, where percentage control growth reached −64.1 ± 0.90%. For the MDA-MB-231 human breast cancer cell line, the positive control Adriamycin (ADR) exhibited strong growth inhibitory activity with GI_{50} and TGI values <10 µg/mL, while the LC_{50} value was observed at 46 µg/mL. These results indicate progressive enhancement of cytotoxicity with increasing drug concentration.

Table 19: Effect of Adriamycin (ADR) on MDA-MB-231 Cell Line

Concentration (µg/mL)	% Control Growth of MDA-MB-231 Cells
10	-34.4 ± 1.80
20	-32.9 ± 3.60
40	-53.5 ± 10.40
80	-64.1 ± 0.90

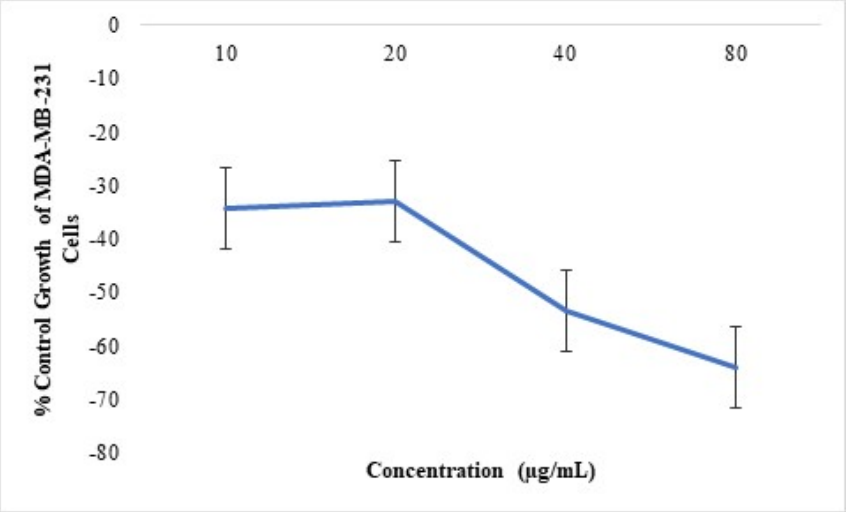
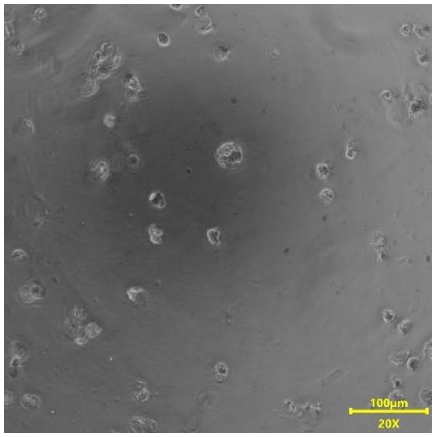


Figure 20: % Control Growth of MDA-MB-231 Cell Line against Adriamycin (ADR) concentration



MDA-MB-231 - ADR

Figure 21: Effect of Adriamycin (ADR) on MDA-MB-231 Cell Line

Statistical Analysis

Table 20: One-way ANOVA for % Control Growth of MDA-MB-231 Cells treated with ADR

Source of Variation	Sum of Squares	df	Mean Square	F value	p-value
Between Groups	2069.49	3	689.82	22.04	< 0.001
Within Groups	250.34	8	31.30	—	—
Total	2319.82	11	—	—	—

One-way ANOVA revealed a statistically significant effect of Adriamycin (ADR) concentration on the % control growth of MDA-MB-231 cells ($F(3,8) = 22.044$, $p < 0.001$), indicating that ADR significantly inhibits cell growth in a concentration-dependent manner, demonstrating strong cytotoxic activity against MDA-MB-231 cells (Table 20).

3.3 Selectivity Index Analysis

To evaluate the selective anticancer potential of the methanolic extract of *Balanites aegyptiaca* leaves, the Selectivity Index (SI) was calculated by comparing cytotoxicity toward normal and cancer cell lines. The SI value provides an indication of preferential toxicity toward malignant cells relative to normal cells. Generally, SI values greater than 2 are considered indicative of selective anticancer activity.

The extract demonstrated comparatively greater toxicity toward cancer cells than normal cells, supporting its selective anticancer potential. The higher selectivity observed against A549 and MCF-7 cell lines suggests that the extract may possess therapeutic advantages with reduced toxicity toward normal tissues.

4. DISCUSSION

The present study evaluated the cytotoxic potential of *Balanites aegyptiaca* leaf extract against multiple human cancer cell lines including cervical cancer (HeLa), lung cancer (A549 and Hop-62), and breast cancer (MCF-7 and MDA-MB-231) using in-vitro cell viability assays such as MTT and SRB. This investigation systematically explored the cytostatic parameters of a methanolic leaf extract of *Balanites aegyptiaca* against a diverse panel of human neoplastic models, including cervical (HeLa), pulmonary (A549, Hop-62), and mammary (MCF-7, MDA-MB-231) cell lines. The primary data established an inverse relationship between extract dose and cellular survival, confirming that the botanical fraction holds broad-spectrum antiproliferative properties. This sweeping reduction in cell viability implies that secondary metabolites inside the leaf matrices are likely interacting with core signal transduction cascades that govern malignant cell cycle progression or survival mechanisms. These observations are closely aligned with historical literature where distinct *Balanites aegyptiaca* preparations demonstrated prominent cytostatic profiles against breast, lung, and prostate models, corroborating the oncological relevance of this species (Ibrahim et al., 2022; Shinde et al., 2025).

The pronounced efficacy noted against HeLa cervical structures points toward an extraction profile capable of triggering programmed cell death or inducing irreversible mitotic arrest. Because of their hyperproliferative characteristics and well-documented sensitivity profiles to natural small molecules, HeLa lineages are highly favored in preliminary screening protocols. The dose-responsive trends observed here match older scientific observations indicating that natural phytochemical compounds can drastically weaken cervical cancer lines by triggering apoptotic signaling cascades and impeding crucial metabolic cycles.

The antiproliferative behavior recorded in the HeLa cervical carcinoma line suggests that this crude leaf extract directly compromises cell division mechanics or triggers internal apoptotic signals. Preclinical screenings regularly employ HeLa lineages due to their highly uniform growth parameters, aggressive replication rates, and reliable sensitivity profiles to novel natural metabolites. The concentration-dependent growth restrictions observed in this study align closely with established literature demonstrating that botanical small molecules reduce cervical cancer cell numbers by altering mitochondrial potential and blocking cell survival metabolic loops. Identical cytotoxic trends occur when testing diverse medicinal plant extracts against HeLa cultures via tetrazolium reduction assays, proving that raw secondary plant metabolites remain an important repository for identifying active structures in cancer drug development (Haridas, 2016).

In this study, the methanolic fraction reduced survival indices across both the A549 and Hop-62 lung cancer lines, expanding the known antineoplastic profile of *Balanites aegyptiaca*. The A549 cell line serves as a standard model for checking epithelial lung tumor responses due to its highly mapped molecular pathways. Prior investigations indicate that purified steroidal saponins isolated from this plant induce classic apoptotic markers, including cell volume loss, cell membrane blistering, and final detachment from the culture surface. These phytochemical variants also stop cell proliferation by halting internal ATP synthesis and disrupting structural tubulin organization (Gnoula et al., 2008).

Parallel cytotoxic effects occurred in the breast cancer models MCF-7 and MDA-MB-231, which is notable because these lines reflect entirely different molecular pathologies. While MCF-7 cells retain estrogen receptor pathways and show less invasive behavior, MDA-MB-231 cells represent triple-negative profiles that generally resist standard cytotoxic regimes. Because cell death occurred across both lines, the active plant constituents likely function independently of classical hormone receptor signaling networks. This multi-lineage growth inhibition corroborates previous data where various *Balanites*

aegyptiaca preparations displayed significant toxicity against mammary carcinomas (Hassan et al., 2016; Ibrahim et al., 2022).

A critical parameter of this in vitro evaluation was the comparative tracking of normal cell responses using human lung fibroblasts (MRC-5), healthy breast epithelium (MCF-10A), and human dermal fibroblasts (HDF). The raw extract exerted significantly less structural damage on these healthy baselines than on the matching malignant cultures, establishing a favorable safety margin. Achieving preferential toxicity against mutated lineages is a primary goal in drug discovery, as it indicates a reduced likelihood of inducing acute side effects in normal tissues. This safety profile stems from differences in baseline metabolic activity and distinct intracellular oxidative stress management between healthy and malignant cell systems (Ibrahim et al., 2022).

When the responses of different cancer cell lines are compared, it becomes evident that the extract exhibits broad-spectrum anticancer activity across multiple tumor types. However, variations in sensitivity among the cell lines were observed. These differences may be attributed to variations in genetic background, receptor expression, metabolic activity, and intracellular signaling pathways between the different cancer models. For example, lung cancer cells such as A549 possess distinct molecular features compared with breast cancer cell lines such as MCF-7 and MDA-MB-231, which may influence their susceptibility to cytotoxic compounds. Such variability is commonly reported in anticancer screening studies involving multiple cancer cell lines (Barretina et al., 2012; Shoemaker, 2006; Skehan et al., 1990).

The observed anticancer effects may be attributed to the phytochemical constituents of *Balanites aegyptiaca*, particularly steroidal saponins such as balanitin-6 and balanitin-7. These compounds have been shown to exhibit strong antiproliferative activity against several cancer cell lines including lung cancer models. Studies suggest that these saponins may disrupt cellular ATP levels, alter cytoskeletal organization, and ultimately impair cancer cell proliferation and migration. Additionally, the extract contains fatty acids, sterols, and other bioactive compounds that may contribute to oxidative stress modulation and activation of apoptotic signalling pathways in cancer cells (Gnoula et al., 2008; Ibrahim et al., 2022).

When compared to the reference anthracycline Adriamycin (ADR), the leaf extract offered a lower absolute potency but an improved selectivity margin. While ADR completely eradicated both normal and malignant cells at doses below 10 µg/mL due to its aggressive DNA-intercalating mechanism, the *Balanites aegyptiaca* leaf extract selectively spared normal cells while progressively killing the tumor cells in a dose-dependent manner. These findings highlight how natural botanical extracts can offer a safer therapeutic window, balancing lower absolute toxicity with selective tumor clearance (Carvalho et al., 2009; Newman & Cragg, 2020).

The IC_{50} values obtained in the present investigation demonstrated differential sensitivity among the tested cancer cell lines. A549 lung cancer cells showed the highest sensitivity toward the methanolic extract of *Balanites aegyptiaca* leaf, as indicated by the lowest IC_{50} value (39.7 µg/mL), suggesting strong susceptibility to the cytotoxic effects of the extract. MCF-7 breast cancer cells also exhibited considerable sensitivity with an IC_{50} value of 48.7 µg/mL. In contrast, HeLa cervical cancer cells displayed comparatively lower sensitivity, with a substantially higher IC_{50} value of 152.1 µg/mL, indicating relative resistance. These variations in responsiveness may be associated with differences in cellular metabolism, membrane composition, oxidative stress tolerance, apoptotic signalling pathways, and genetic characteristics among different cancer cell types.

The statistical analysis performed across both MTT and SRB assays confirmed the reliability and significance of the observed cytotoxic effects of *Balanites aegyptiaca* leaf extract. Two-way ANOVA analysis of the MTT assay data for lung (A549 vs. MRC-5) and breast (MCF-7 vs. MCF-10A) cell lines revealed highly significant effects of concentration and cell type ($p < 0.001$), as well as significant interaction effects between these factors. This indicates that the cytotoxic response varied depending on both the extract concentration and the biological characteristics of the cell lines. Similarly, one-way ANOVA analyses performed for the HeLa and HDF cell lines demonstrated statistically significant differences in cell viability across concentrations ($p < 0.001$), confirming a strong dose-dependent effect of the extract. Comparable statistical trends were observed in the SRB assay, where significant differences in percentage control growth were detected for HeLa, Hop-62, and MDA-MB-231 cell lines ($p < 0.001$). These results collectively demonstrate that the observed reduction in cell viability and proliferation was not due to random variation but represents a reproducible and concentration-dependent cytotoxic effect of *Balanites aegyptiaca* leaf. The consistent statistical significance across multiple cancer cell lines further strengthens the validity of the extract's antiproliferative activity and supports its potential as a promising natural source of anticancer compounds.

5. CONCLUSION

The present study demonstrated that the methanolic leaf extract of *Balanites aegyptiaca* possesses significant dose-dependent cytotoxic and antiproliferative activity against multiple human cancer cell lines, including lung, breast, and cervical cancer cells. The extract showed comparatively greater toxicity toward malignant cells than normal cell lines, indicating promising selective anticancer potential and a comparatively safer biological profile. Among the tested cell lines, lung and breast cancer cells exhibited higher sensitivity to the extract, whereas normal cells maintained relatively higher viability. The observed anticancer activity may be associated with the presence of

bioactive phytoconstituents such as steroidal saponins, flavonoids, and phenolic compounds, which are known to induce apoptosis and inhibit cancer cell proliferation. Morphological changes observed in treated cells further supported cytotoxic activity. The findings suggest that *Balanites aegyptiaca* may serve as a valuable natural source for future anticancer drug development. However, further mechanistic and in vivo studies are required to confirm its therapeutic potential.

Acknowledgement
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REFERENCES

1. Abdelaziz, S. M., Lemine, F. M. M., Tfeil, H. O., Filali-Maltouf, A., & Boukhary, A. O. M. S. (2020). Phytochemicals, Antioxidant Activity and Ethnobotanical Uses of Balanites aegyptiaca (L.) Del. Fruits from the Arid Zone of Mauritania, Northwest Africa. Plants 2020, Vol. 9, 9(3). https://doi.org/10.3390/plants9030401
2. Alley, M. C., Scudiero, D. A., Monks, A., Hursey, M. L., Czerwinski, M. J., Fine, D. L., Abbott, B. J., Mayo, J. G., Shoemaker, R. H., & Boyd, M. R. (1988). Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Research, 48(3), 589–601.
3. Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A. A., Kim, S., Wilson, C. J., Lehár, J., Kryukov, G. V., Sonkin, D., Reddy, A., Liu, M., Murray, L., Berger, M. F., Monahan, J. E., Morais, P., Meltzer, J., Korejwa, A., Jané-Valbuena, J., … Garraway, L. A. (2012). The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature, 483(7391), 603–607. https://doi.org/10.1038/nature11003
4. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 68(6), 394–424. https://doi.org/10.3322/caac.21492
5. Carvalho, C., Santos, R., Cardoso, S., Correia, S., Oliveira, P., Santos, M., & Moreira, P. (2009). Doxorubicin: The Good, the Bad and the Ugly Effect. Current Medicinal Chemistry, 16(25), 3267–3285. https://doi.org/10.2174/092986709788803312
6. Chothani, D. L., & Vaghasiya, H. U. (2011). A review on Balanites aegyptiaca Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity. Pharmacognosy Reviews, 5(9), 55. https://doi.org/10.4103/0973-7847.79100
7. Gnoula, C., Mégalizzi, V., De Nève, N., Sauvage, S., Ribaucour, F., Guissou, P., Duez, P., Dubois, J., Ingrassia, L., Lefranc, F., Kiss, R., & Mijatovic, T. (2008). Balanitin-6 and -7: diosgenyl saponins isolated from Balanites aegyptiaca Del. display significant anti-tumor activity in vitro and in vivo. International Journal of Oncology, 32(1), 5–15.
8. Gordon, J. L., Brown, M. A., & Reynolds, M. M. (2018). Cell-Based Methods for Determination of Efficacy for Candidate Therapeutics in the Clinical Management of Cancer. Diseases, 6(4), 85. https://doi.org/10.3390/diseases6040085
9. Haridas, R. (2016). In-vitro cytotoxicity activity of malaxis rheedei sw methanol extract against hela cell line and mcf- 7 cell line. Asian Journal of Pharmaceutical and Clinical Research, 9(6), 244. https://doi.org/10.22159/ajpcr.2016.v9i6.14298
10. Hassan, L. E. A., Dahham, S. S., Saghir, S. A. M., Mohammed, A. M. A., Eltayeb, N. M., Majid, A. M. S. A., & Majid, A. S. A. (2016). Chemotherapeutic potentials of the stem bark of Balanite aegyptiaca (L.) Delile: an antiangiogenic, antitumor and antioxidant agent. BMC Complementary and Alternative Medicine, 16(1), 396. https://doi.org/10.1186/s12906-016-1369-5
11. Holohan, C., Van Schaeybroeck, S., Longley, D. B., & Johnston, P. G. (2013). Cancer drug resistance: an evolving paradigm. Nature Reviews Cancer 2013 13:10, 13(10), 714–726. https://doi.org/10.1038/nrc3599
12. Hosseini, A., & Ghorbani, A. (2015). Cancer therapy with phytochemicals: evidence from clinical studies. Avicenna Journal of Phytomedicine, 5(2), 84.
13. Ibrahim, O. H. M., Al-Qurashi, A. D., Asiry, K. A., Mousa, M. A. A., Alhakamy, N. A., & Abo-Elyousr, K. A. M. (2022). Investigation of Potential In Vitro Anticancer and Antimicrobial Activities of Balanites aegyptiaca (L.) Delile Fruit Extract and Its Phytochemical Components. Plants, 11(19), 2621. https://doi.org/10.3390/plants11192621
14. Juthani, R., Punatar, S., & Mittra, I. (2024). New light on chemotherapy toxicity and its prevention. BJC Reports 2024 2:1, 2(1), 41-. https://doi.org/10.1038/s44276-024-00064-8
15. Lovitt, C. J., Shelper, T. B., & Avery, V. M. (2013). Miniaturized Three-Dimensional Cancer Model for Drug Evaluation. ASSAY and Drug Development Technologies, 11(7), 435–448. https://doi.org/10.1089/adt.2012.483
16. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65(1–2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4
17. Murthy, H. N., Yadav, G. G., Dewir, Y. H., & Ibrahim, A. (2020). Phytochemicals and Biological

Activity of Desert Date (Balanites aegyptiaca (L.) Delile). Plants (Basel, Switzerland), 10(1), 1–22. https://doi.org/10.3390/plants10010032

18. Newman, D. J., & Cragg, G. M. (2020). Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. Journal of Natural Products, 83(3), 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285
19. Orellana, E., & Kasinski, A. (2016). Sulforhodamine B (SRB) Assay in Cell Culture to Investigate Cell Proliferation. Bio-Protocol, 6(21). https://doi.org/10.21769/bioprotoc.1984
20. Reyaz, A., Qadri, S. S., Javaid, D., Ganie, S. Y., & Reshi, M. S. (2025). Exploring the role of traditional medicinal plants in cancer therapy: present efficacy and future directions. Phytomedicine Plus, 5(3), 100845. https://doi.org/10.1016/j.phyplu.2025.100845
21. Sharma, J. (2011). A Review Report on cancer treatment.
22. Sharma, S. V., Haber, D. A., & Settleman, J. (2010). Cell line-based platforms to evaluate the therapeutic efficacy of candidate anticancer agents. Nature Reviews Cancer, 10(4), 241–253. https://doi.org/10.1038/nrc2820
23. Shinde, A. S., Ingole, V. V., Rasale, S. P., & Katade, S. R. (2025). Balanites aegyptiaca (L.) Delile: a review of its traditional uses, phytochemistry and biological activities. Phytomedicine Plus, 5(3), 100848. https://doi.org/10.1016/j.phyplu.2025.100848
24. Shoemaker, R. H. (2006). The NCI60 human tumour cell line anticancer drug screen. Nature Reviews Cancer, 6(10), 813–823. https://doi.org/10.1038/nrc1951
25. Singh, D., Vignat, J., Lorenzoni, V., Eslahi, M., Ginsburg, O., Lauby-Secretan, B., Arbyn, M., Basu, P., Bray, F., & Vaccarella, S. (2023). Global estimates of incidence and mortality of cervical cancer in 2020: a baseline analysis of the WHO Global Cervical Cancer Elimination Initiative. The Lancet Global Health, 11(2), e197–e206. https://doi.org/10.1016/S2214-109X(22)00501-0
26. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., & Boyd, M. R. (1990). New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. JNCI Journal of the National Cancer Institute, 82(13), 1107–1112. https://doi.org/10.1093/jnci/82.13.1107
27. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 71(3), 209–249. https://doi.org/10.3322/caac.21660
28. Vichai, V., & Kirtikara, K. (2006). Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature Protocols, 1(3), 1112–1116. https://doi.org/10.1038/nprot.2006.179
29. Was, H., Borkowska, A., Bagues, A., Tu, L., Liu, J. Y. H., Lu, Z., Rudd, J. A., Nurgali, K., & Abalo, R. (2022). Mechanisms of Chemotherapy-Induced Neurotoxicity. Frontiers in Pharmacology, 13, 750507. https://doi.org/10.3389/fphar.2022.750507