

Moringa oleifera Leaf Extract-Mediated Suppression of Epithelial to Mesenchymal Transition

Samrat Paul¹, Anuradha Moirangthem², Nilanjana Ghosh³, Sohini Sen⁴, Sanjib Ray², Piyali Basak^{3,*}, Anupam Basu²

¹Department of Biotechnology, Brainware University, Barasat, Kolkata, West Bengal, INDIA.

²Department of Zoology, Molecular Biology and Human Genetics Laboratory, The University of Burdwan, Burdwan, West Bengal, INDIA.

³Department of Biomedical Engineering, School of Bioscience and Engineering, Jadavpur University, Kolkata, West Bengal, INDIA.

⁴Basic Science and Humanities, Institute of Engineering and Management, University of Engineering and Management, Kolkata, West Bengal, INDIA.

ABSTRACT

Background: The increasing incidence of breast cancer remains a major global health challenge despite notable progress in diagnosis and therapy. Many patients progress to metastatic stages, where current treatments are not only costly but often less effective. This has emphasized the need to identify affordable alternative with anti-metastatic potential. *Moringa oleifera*, widely recognized for its cancer-preventative potential, was the focus of this study that explore its anti-metastatic properties. **Aim:** This study aimed to evaluate the anti-metastatic potential of methanolic extract of *Moringa oleifera* and elucidate its underlying molecular mechanisms against metastatic breast cancer cell lines. **Materials and Methods:** Methanolic extract of *Moringa oleifera* leaves was prepared and tested on epithelial (MCF-7) and mesenchymal (MDA-MB-231) breast cancer cell lines. Cell adhesion assays on gelatin-fibronectin-coated plates and migration assays were conducted to assess metastatic behavior. Flow cytometry was used for cell cycle analysis, and GC-MS was employed to identify bioactive compounds. Expression of EMT-related markers and caspase-3 activation were evaluated to determine apoptotic and mechanistic pathways. **Results:** Treatment with 25 µg/mL methanolic *Moringa oleifera* extract significantly inhibited proliferation of MDA-MB-231 cells more effectively than MCF-7 cells, primarily through caspase-3-dependent apoptosis. The extract induced cell cycle arrest at the G0/G1 phase in MCF-7 and G2/M phase in MDA-MB-231 cells, indicating growth suppression. GC-MS analysis identified benzyl isocyanate as a major bioactive compound, which likely inhibited the Slug transcription factor, upregulated CDH1 expression, and may enhanced E-cadherin levels, thereby preventing Epithelial to Mesenchymal Transition (EMT). **Conclusion:** Methanolic extract of *Moringa oleifera*, rich in benzyl isocyanate, effectively suppresses mesenchymal transition and metastatic progression in breast cancer cells through apoptosis induction, adhesion reduction, and migration inhibition. These findings highlight its promise as a cost-effective therapeutic candidate for preventing breast cancer metastasis.

Keywords: Breast Cancer Metastasis, Anti-metastatic Properties, *Moringa oleifera*, Apoptotic Pathway, Epithelial to Mesenchymal Transition (EMT), Benzyl Isocyanate.

Correspondence:

Dr. Piyali Basak

Associate professor, School of Bioscience and Engineering, Jadavpur University, Kolkata-700032, West Bengal, INDIA.
Email: piyalibasak@gmail.com

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INTRODUCTION

Cancer is a significant health concern in West Bengal, India. Data from national and regional cancer centers from 1984 to 2002 have shown an increasing incidence of breast cancer.¹ National Cancer Registries from Regional Cancer Centers indicate that breast cancer is particularly common among women in Delhi, Mumbai, Ahmedabad, Kolkata, and Trivandrum.² A population-based

cancer registry in Kolkata suggests that many breast cancer diagnoses occur at the metastatic stage.¹ Metastasis happens when epithelial cancer cells lose their apico-basal polarity and adopt a spindle-shaped morphology. This transition is often driven by carcinogen-induced mutations in the CDH1 gene, which inactivate E-cadherin, leading to reduced cell-to-cell adhesion and cell matrix adhesion.³

Moringa oleifera, a medicinal herb traditionally used in India, has shown promise in cancer prevention. It has been reported to inhibit tumor promotion in a mouse two-stage DMBA-TPA tumor model and prevent skin cancer.^{4,5} Studies have also established that *Moringa oleifera* leaf extract inhibits cell proliferation and induces apoptosis in human KB tumorigenic cell lines and A549 lung cells through NF-κB mediated signaling pathways.⁶⁻⁸



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Given these findings, our research aimed to evaluate the anti-metastatic activity of *Moringa oleifera* leaf extract specifically against breast cancer. We treated MCF-7 (epithelial) and MDA-MB-231 (mesenchymal) breast cancer cell lines with the extract and assessed various parameters to determine its efficacy as a chemo-preventive therapeutic agent.

MATERIALS AND METHODS

Leaf extracts preparation

Moringa oleifera leaves, approved by the Central National Herbarium, Botanical Survey of India (CNH-BSI), were extracted using solvents including petroleum ether, chloroform, distilled water, and methanol, as described in our previous publication.⁹ The extracts were lyophilized and mixed with 0.25 µmol/L filtered sterilized DMSO (500 µL for 10 mg) before storage at -20°C.¹⁰ Working solutions were prepared via serial dilution of the stock.

Antioxidant Activity Assessment Using the DPPH Assay

The antioxidant activity was assessed using the methanolic extract. 0.1 mM DPPH (SRL) solution was prepared in methanol. For the experiment, 4 mL of DPPH served as the control, while 1 mL DPPH mixed with 3 mL extract constituted the sample solution. 4 mL extract sample was used as a blank. After vigorous shaking and a 30-min incubation at room temperature, antioxidant activity was measured at 517 nm using an Agilent Technologies Cary 60 UV-vis Spectrophotometer. (Figure S1) L-ascorbic acid standard curves (1-0.625 µg/mL) were used for comparison, and antioxidant activity was calculated using formula 1.

$$AA\% = 100 - \left[\frac{(\text{Abs Sample} - \text{Abs Blank})}{\text{Abs control}} \right] \times 100 \dots\dots 1$$

GC-MS analysis

GC-MS analysis of the *Moringa oleifera* leaf extract was performed using an Ion Trap technology on a Thermo Scientific Polaris-Q mass spectrometer. The temperature program started at 70°C for 3 min, increased at 10°C/min to 240°C, and ended with a 9-min isothermal hold at 280°C. 1 µL sample was manually injected into a DB5MS silica capillary column (30 m x 0.25 mm ID x 0.25 µm), with a 10:1 split ratio, at an injector temperature of 240°C and ion source temperature of 220°C. Helium (99.999%) served as the carrier gas at 1 ml/min, operating in electron impact mode at 70 eV. The detector scanned from 50 to 950 m/z over 40 min, with data stored using X-caliber software. Compound identification was based on mass spectral matching with the NIST 2014 library.

Cell culture

Cancer cell line culture

MCF-7 (epithelial breast cancer), MDA-MB-231 (mesenchymal breast cancer), and HeLa (cervical cancer) cell lines were procured from NCCS Pune, India. HeLa cells were used for

IC₅₀ screening, while MCF-7 and MDA-MB-231 were employed to evaluate anti-metastatic activity. Cells were cultured with DMEM (Himedia) growth medium supplemented with 10% FBS (Himedia), and 2% L-Glutamine-Penicillin-Streptomycin (Himedia) as per standard protocol.

PBMCs culture

Ethical approval for blood collection from healthy donors was granted by Jadavpur University's Institutional Bioethics Committee. 3 mL of blood were collected in EDTA vials and PBMCs isolated using density gradient centrifugation with Lymphoprep (Fresenius Kabi Norge AS). Cells were washed, resuspended in RPMI-1640 (Himedia) complete media, and incubated at 37°C with 5% CO₂.

Cell viability and cytotoxicity assay

MTT assays on HeLa, MCF-7, MDA-MB-231, and PBMCs pre- and post-leaf extract treatment identified the most effective dose by inhibitory capacity. The statistically significant dose was chosen, with IC₅₀ values measured for HeLa and PBMCs,¹¹ and the absorbance at 590 nm after 3 hr determined cell viability percentages.

Evaluating Anti-Metastatic Activity via Cell Adhesion and Migration Assays

Twelve-well plates were coated with diluted gelatin (250 µg/mL) and incubated at 37°C for 1.5 hr. The polymerized gelatin surface was then modified with fibronectin solution (2 µg/mL in PBS). MCF-7 and MDA-MB-231 cells were seeded in the Gelatin-Fibronectin (GF) coated plates at a density of 10x10⁴ in 1 mL of complete media.^{12,13} After 6 hr, the media was removed and non-adherent cells were washed off. MTT treatment was conducted in the no-treatment group to measure cell adhesion percentage using formula 2.

$$\% \text{ of cell adhesion} = \left[\frac{\text{Number of cell by MTT}}{\text{Number of cell seeded}} \right] \times 100 \dots\dots 2$$

Cell numbers were calculated using a regression equation obtained from a standard curve of OD values vs seeded cell numbers. Leaf extract was added to the treatment group and incubated for 24 hr, followed by MTT treatment to assess cell adhesion, death, and anti-metastatic activity.

The same procedure was applied to 25mm² dishes where the cells adhered to gelatin-fibronectin coated sterile coverslips. Adherent cells were fixed with 100% chilled methanol, washed with FBS in PBS (1:1 v/v), and then stained with DAPI followed by incubation for 10 min in the dark at 4°C. Observations were made using a fluorescent microscope with a DAPI filter at 360 nm.¹⁴

A Scratch wound heal assay quantified cellular migration in two-dimensional monolayer cultures. Cells were seeded in 24-well plates at a density of 25 x 10⁴ cells/well in 500 µL of complete media. Upon reaching 80-90% confluence, a scratch

was made using a sterile pipette. Following leaf extract treatment, micrographs of the scratch were taken at 0 hr, 24 hr, and 48 hr with a phase-contrast microscope. The cell migration rate was calculated based on the time required to close 50% of the gap.¹⁵

Cell Death Mechanisms: Flow Cytometry and Immunoblotting Analysis of Apoptosis and Cell Cycle Regulation

Flow cytometry was performed using the BD FACS Aria to identify treatment-induced apoptosis and necrosis. Adherent cells were harvested, washed with ice-cold PBS, and stained with the Annexin V/PI apoptosis kit (Biovision Inc; K101-25). After a 20-min incubation in the dark, fluorescence emission at 530 nm and 575 nm was measured using 488 nm excitation.

Cells were washed with ice-cold PBS containing 2% FBS and fixed with 80% chilled ethanol added dropwise with vortexing, followed by a 30-min incubation. Fixed cells were treated with Ribonuclease A (Sigma Aldrich) for 30 min at 4°C in the dark, stained with Propidium Iodide (Sigma-Aldrich), and incubated again for 30 min at 4°C in the dark.¹⁴ Cell cycle and DNA content were analyzed using the BD FACSVerse.

A Caspase-3 immunoblotting assay was conducted using an ELISA kit (Elabsience; cat no: E-EL-H0017) according to the provided protocol to investigate apoptosis pathways.

Data archiving and analysis

The experiment was repeated three times, with data recorded. One-way ANOVA between control and treated groups was conducted using Origin 6.1 software. Results were displayed as line diagrams or bar charts with mean $\pm$ SD. Significant differences ($p<0.05$) at a 95% confidence interval were calculated and represented alphabetically.

RESULTS AND DISCUSSION

Moringa oleifera leaf extract contains polyphenolic and flavonoid compounds that trap reactive oxygen species and other free radicals. The total alkaloid content measured was 0.0891 $\pm$ 0.02355 mg/100g of leaf extract. Its anti-gonadotrophic hormonal activity supports the prevention of breast cancer cell progression. Various

solvents were used to prepare the leaf extract, but the pet-ether and methanolic extracts showed significant eradication ($p<0.05$; $n = 6$) compared to controls and the standard positive control Cis-platin (16.66 μ g/mL).

The pet-ether extract formed a sticky, clay-like material due to incomplete solvent removal, causing cell toxicity.¹⁶ Thus, the methanol extract was selected for further experiments, as it demonstrated significant inhibition ($p<0.05$; $n= 6$) of HeLa cells compared to other solvent extracts.

The DPPH antioxidant activity of the methanolic leaf extract was estimated at 73.03%, comparable to 0.78 mg/dL of L-ascorbic acid in free radical scavenging activity. The IC₅₀ value of the methanolic leaf extract, determined by dose-response curves on HeLa cells and PBMcs, was already peer-reviewed and published (Figure S2) (Paul *et al.*, 2019). Both 50 μ g/mL and 25 μ g/mL concentrations significantly inhibited HeLa cell growth compared to normal PBMcs (Figure S3 and Table 2). However, the 25 μ g/mL extract showed less cytotoxicity to normal cells ($p<0.05$; $n = 6$) and was selected for further experiments. A single-dose study on MCF-7, MDA-MB-231, and PBMcs revealed that the 25 μ g/mL extract was more effective on MDA-MB-231 cells than on MCF-7 cells (Figure S4). This dose also reduced cell proliferation rates for both cell lines over 24 to 48 hr.

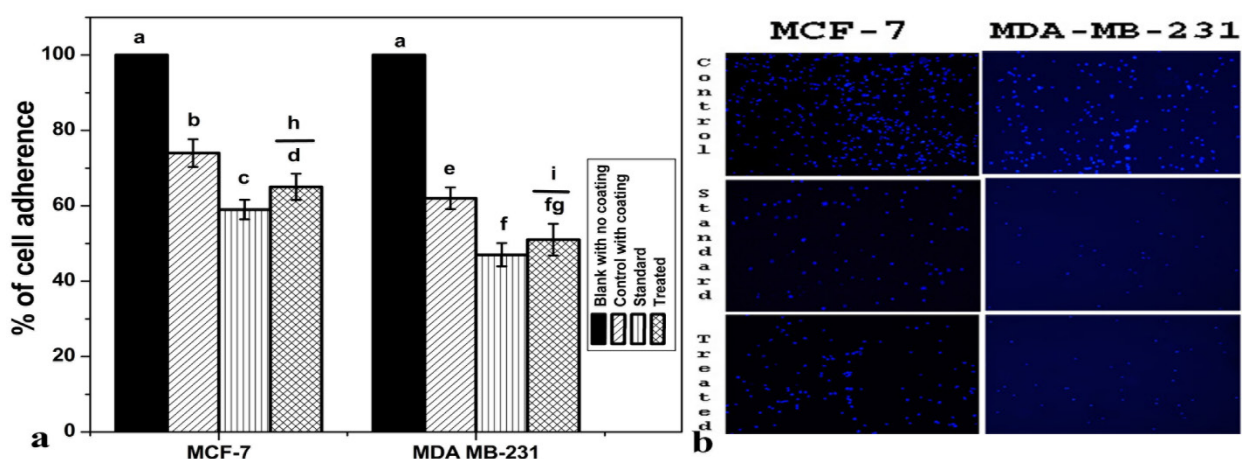
The Epithelial-to-Mesenchymal Transition (EMT) involves significant changes in cellular morphology, including reduced cell-to-cell and cell-matrix adhesion, leading to cell polarization and the acquisition of migratory and invasive properties.¹⁷ These properties are crucial for mesenchymal-like activity and metastasis.¹⁸ In our study, we investigated inhibition of *in vitro* metastasis activity using cell-based adhesion and scratch wound healing assays (Figure 1a). Adhesion of MCF-7 (74%) and MDA-MB-231 (62%) cells significantly decreased ($p<0.05$; $n = 3$) on Gelatin-Fibronectin (GF) coated plates compared to normal plates. Treatment with *Moringa oleifera* leaf extract further reduced cell adherence in both lines, showing greater potency equivalent to Cisplatin on MDA-MB-231 cells (Figure 1b). This suggests the extract is more effective against mesenchymal cells compared to epithelial cells.

Table 1: *Moringa oleifera* leaf extract induced flow cytometric analysis for estimation of cell population in different phases of cell cycle measured after propidium iodide stain.

	Phases of cell cycle	Control (Mean $\pm$ SD)	Standard (Mean $\pm$ SD)	Treated (Mean $\pm$ SD)
MCF-7	Go/G1 (P3)	39.78 $\pm$ 1.34	55.86 $\pm$ 2.11	49.29 $\pm$ 1.11
	S (P5)	12.26 $\pm$ 0.96	13.49 $\pm$ 0.65	13.22 $\pm$ 0.96
	G2/M (P4)	24.57 $\pm$ 2.24	9.15 $\pm$ 0.75	8.23 $\pm$ 0.86
MDA-MB-231	Go/G1 (P3)	70.78 $\pm$ 2.96	59.64 $\pm$ 2.98	65.49 $\pm$ 2.12
	S (P5)	10.6 $\pm$ 0.89	7.29 $\pm$ 0.68	7.98 $\pm$ 0.89
	G2/M (P4)	10.79 $\pm$ 1.06	20.94 $\pm$ 2.12	18.31 $\pm$ 1.65

Table 2: GC-MS chromatogram peak analysis of the Leaf extract and the possible name of the compound after comparing the peak value with NIST library version 2014.

Apex RT	Area	Height	Name of the compound as per NIST library
16.92	924.462	205.986	4 ethyl 2 hexanal
17.18	4411.928	841.655	phthalic acid,2 phenyl ethyl propyl ester
17.9	1484.003	321.411	Benzene propanoic acid, 3,5 bis(1,1-dimethylethyl)-4 hydroxy, methyl ester
19.61	3202.077	467.135	Cyclopentane, 1 isobutylidene-3-methyl
23.71		4934.564	benzyl Isocyanate
24.03	6293.067	1195.147	Hexadenoic acid,2-hydroxy -1(hydroxymethyl)ethyl ester
24.24	2467.209	378.914	1,3 dichloro 2 propanol
24.48	5275.115	934.875	Adipic acid, 3 methylbut-3 ethyl propyl ester
25.84	21987.91	2862.817	Methyl 8, 11, 14, 17 eicosatetraenoate
31.19	7328.965	1257.184	Tocopherol-o-methyl
34.96	11716.01	2249.192	Sitosterol

**Figure 1:** a) Treatment with *Moringa oleifera* leaf extract was evaluated using a cell-based adhesion assay. All data were expressed as Mean $\pm$ SD. b) DAPI-stained microphotograph showing adherent cells.

DAPI staining of control, standard, and treated cells followed by fluorescence microscopy complemented the spectrophotometric results. DAPI (4',6-diamidino-2-phenylindole) binds to A=T regions in fixed cell DNA, enabling cell nuclei counting and overall morphology assessment. The adhesive capacity of MCF-7 and MDA-MB-231 cells decreased post-treatment, with fewer cells observed in MDA-MB-231 (Figure 1b). This suggests that *Moringa oleifera* leaf extract may have antimetastatic effects.

Cellular morphology was reviewed under an Olympus inverted phase contrast microscope, and images were captured using a Magnus MIPS USB2.0 camera at 10X zoom. In Figure 2 (a1 & a2), MCF-7 and MDA-MB-231 cells exhibit sharp morphology. A scratch wound was created and recognized in Figure 2 (b1 & b2) and showed that the leaf extract significantly inhibited wound closure within 48 hr.

MCF-7 exhibited a higher migration rate than MDA-MB-231. This result supports the antimetastatic activity. Post-treatment, some cells displayed sharp morphology while others appeared

rounded and shrunken, particularly in MDA-MB-231 (Figure c1 & c2). This morphological change may result from leaf extract-induced apoptosis and cell growth arrest.

Flow cytometry analysis of MCF-7 and MDA-MB-231 cells treated with *Moringa oleifera* leaf extract demonstrated a reduction in cancer cell viability. The number of live cells decreased significantly from 32.80% (control) to 18.72% (treated) for MCF-7, and from 86.52% (control) to 63.59% (treated) for MDA-MB-231, alongside a notable increase in apoptotic cells in both cell lines (Figure 3 (a1 & b1)). Significant changes ($p < 0.05$, $n = 10$) were observed in the percentage of MDA-MB-231 cells in early and late apoptosis, but not in MCF-7 cells (Figure b), compared to the control group.

The extract induced cell growth arrest at the G0/G1 phase for MCF-7 and the G2/M phase for MDA-MB-231 (Figure 3 (a2 & b2)). Table 1 shows an increase in the MCF-7 cell population at the G0/G1 and S phases in treated groups, with a significant rise ($p < 0.05$, $n = 6$) in G0/G1 compared to the control group, indicating

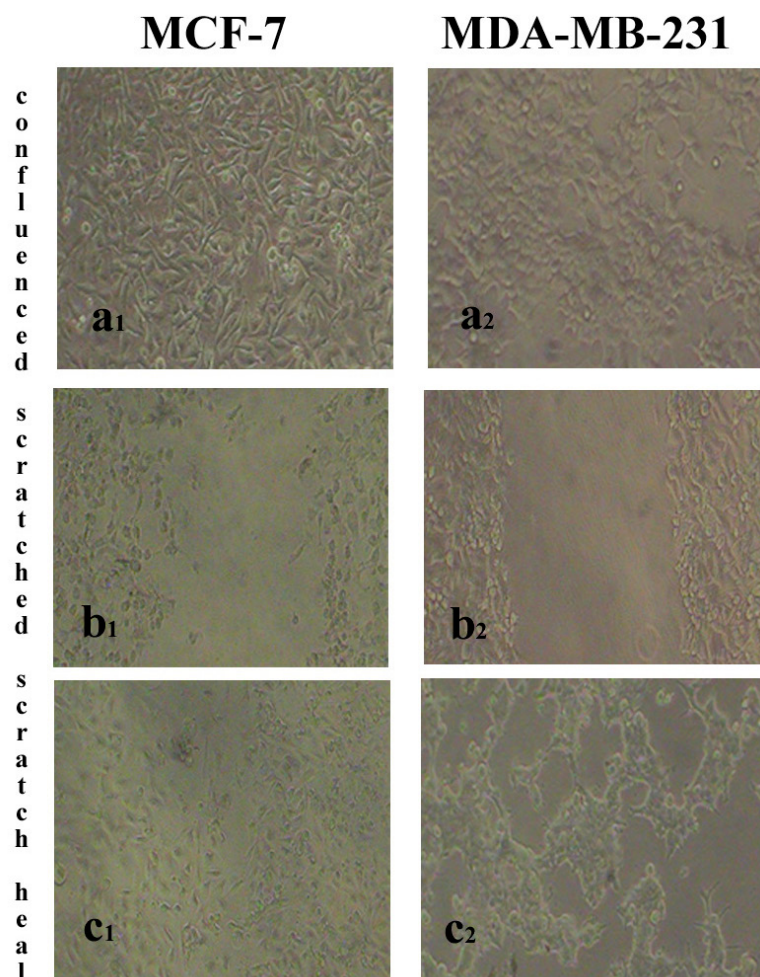


Figure 2: Micro-photographic observation of scratch wound heals cell migration assay after leaf extract treatment.

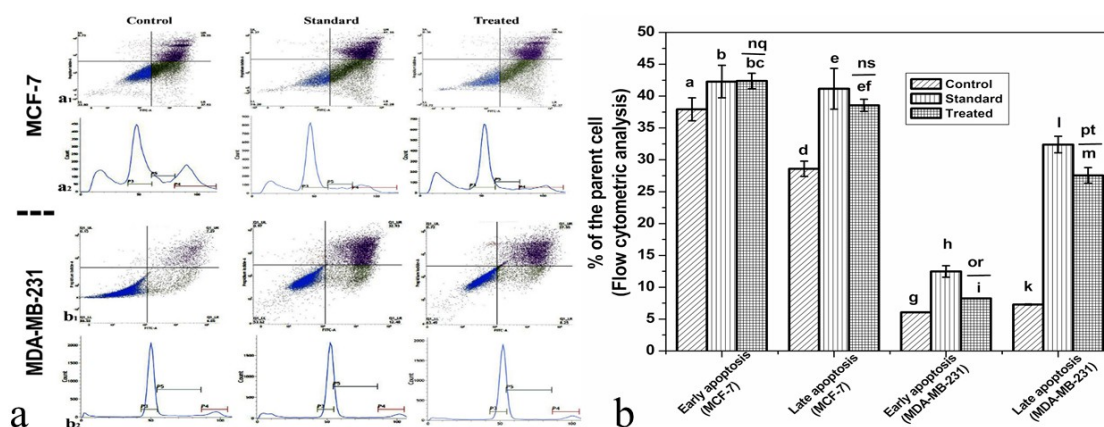


Figure 3: Flow-cytometric analysis was performed to evaluate apoptosis and cell-cycle regulation in MCF-7 and MDA-MB-231 cells following treatment with *Moringa oleifera* leaf extract. The experiment was conducted in triplicate. Data were analyzed using one-way ANOVA. Identical alphabetical letters indicate no significant difference, whereas different letters represent statistically significant differences at $p < 0.05$. The values presented in the figures correspond to the nearest mean values.

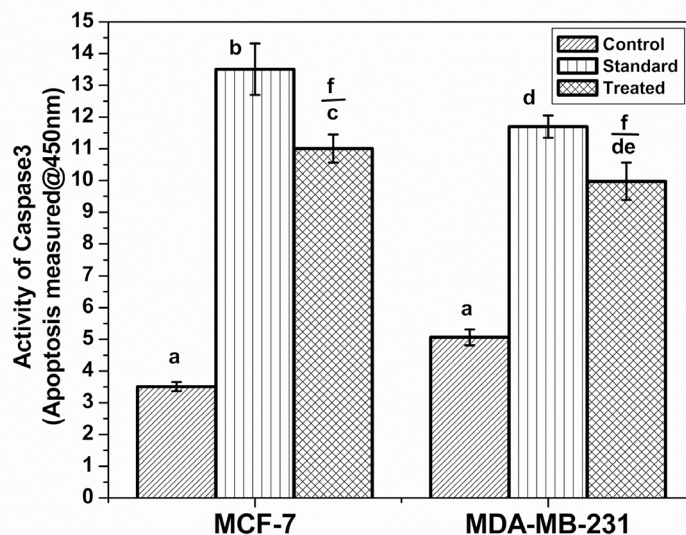


Figure 4: Caspase-3 immunoblotting was performed to confirm the apoptotic pathway induced by the treatment. One-way ANOVA was conducted for statistical analysis. Identical alphabetical letters indicate no significant difference, whereas different letters represent statistically significant differences at $p < 0.05$.

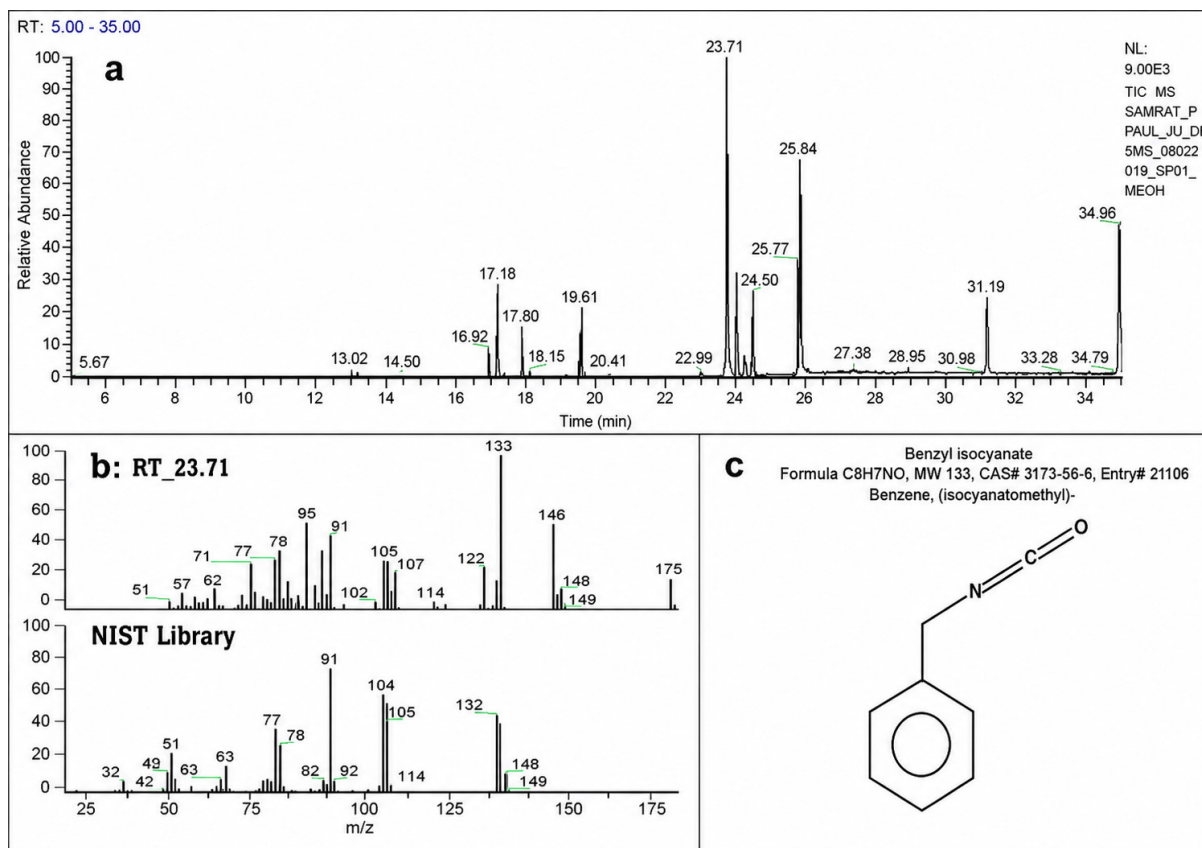


Figure 5: GC-MS Chromatogram analysis of the leaf extract.

cell cycle arrest at this phase. Similarly, the MDA-MB-231 cell population increased in the G₂/M phase with a reduction in G₀/G₁ and S phases, suggesting cell cycle arrest at the G₂/M phase.

An immunoblotting assay for Caspase-3 was conducted to establish the pathway promoting apoptosis.¹⁹ Caspase-3 is a key executor in this process, responsible for degrading chromosomal DNA and

causing chromatin condensation. Figure 4 demonstrated that the leaf extract induced apoptosis in both MCF-7 and MDA-MB-231 cell lines. The results showed a significant effect compared to the untreated control, with no significant difference between the treated groups of MCF-7 and MDA-MB-231. This indicates that the leaf extract inhibited cancer cells and facilitated apoptosis via caspase-dependent pathways.

The GC-MS analysis of the leaf extract identified 11 peaks, as summarized in Table 2 with their corresponding retention times and compounds. The highest peak (4934.564) was observed at a retention time of 23.71, with a relative peak area of 30642.24, corresponding to the compound benzyl isocyanate (Figure 5). This finding is supported by the successful isolation of Bis (isothiocyanatomethyl) benzene (the dimeric form of benzyl isothiocyanate) from the *Moringa oleifera* leaf extract.²⁰ Consequently, it was confirmed that *Moringa oleifera* leaf extract inhibits EMT.

CONCLUSION

Moringa oleifera leaf extract demonstrates substantial potential in inhibiting breast cancer progression. The extract contains polyphenolic and flavonoid compounds that effectively scavenge reactive oxygen species and free radicals. With a measured total alkaloid content of 0.0891 ± 0.02355 mg/100g, the extract shows notable anti-gonadotropic hormonal activity, which supports the prevention of breast cancer cell progression.

The methanolic extract was selected based on its significant cytotoxicity against HeLa cells and minimal toxicity to normal PBMCs. The methanolic extract exhibited strong antioxidant activity, with a DPPH scavenging activity of 73.03%, comparable to that of L-ascorbic acid. It effectively inhibited the growth of HeLa cells, particularly at concentrations of 25 µg/mL, which also showed reduced cytotoxicity to normal cells.

The extract's efficacy was further demonstrated in single-dose studies on MCF-7 and MDA-MB-231 cells, showing a higher effectiveness on MDA-MB-231 cells. The extract reduced cell proliferation rates and demonstrated significant antimetastatic activity by inhibiting Epithelial-to-Mesenchymal Transition (EMT). Adhesion and scratch wound healing assays indicated reduced cell adherence and migration, particularly in MDA-MB-231 cells, suggesting the extract's potential to inhibit metastasis. DAPI staining and fluorescence microscopy confirmed the extract's ability to reduce cancer cell viability and induce apoptosis. Flow cytometry analysis showed a significant decrease in live cell populations and an increase in apoptotic cells, particularly in MDA-MB-231 cells. The extract also induced cell cycle arrest at different phases for the two cell lines, further supporting its anticancer properties.

GC-MS analysis identified benzyl isocyanate as a major compound in the extract, corroborating its inhibitory effect on

EMT. The successful isolation of Bis (isothiocyanatomethyl) benzene further supports this finding. Immunoblotting assays confirmed the activation of caspase-3, indicating that the extract facilitates apoptosis via caspase-dependent pathways.

Overall, *Moringa oleifera* leaf extract exhibits significant anticancer, antioxidant, and antimetastatic properties, making it a promising candidate for further research and development in cancer therapy.

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ABBREVIATIONS

EMT: Epithelial to Mesenchymal Transition; **GC-MS:** Gas Chromatography-Mass Spectrometry; **PBMCs:** Peripheral Blood Mononuclear Cells; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **DAPI:** 4',6-Diamidino-2-phenylindole; **FBS:** Fetal Bovine Serum; **DMSO:** Dimethyl Sulfoxide; **ANOVA:** Analysis of Variance; **SD:** Standard Deviation; **IC₅₀:** Half Maximal Inhibitory Concentration; **ROS:** Reactive Oxygen Species; **NF-κB:** Nuclear Factor Kappa-light-chain-enhancer of Activated B Cells.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

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SUPPLEMENTARY INFORMATION

The IC₅₀ value, Standard Curve, Statistical analysis report and others supportive information's are available as supportive supplementary material.

ETHICAL APPROVAL

The Ethical permission for collection of peripheral blood from healthy donors irrespective of male and female, was obtained from Institutional Bioethics Committee, Bioequivalence Study Centre, Jadavpur University. No animals were used for that study.

SUMMARY

The article investigates the anti-metastatic potential of *Moringa oleifera* leaf extract against breast cancer, focusing on its role in suppressing Epithelial-to-Mesenchymal Transition (EMT). Despite advancements in treatment, breast cancer metastasis remains a major clinical challenge, prompting the search for cost-effective phyto-therapeutic alternatives. Methanolic extracts of *Moringa oleifera* leaves were prepared and tested on epithelial (MCF-7) and Mesenchymal (MDA-MB-231) breast cancer cell lines. Antioxidant evaluation revealed strong free radical scavenging activity (73.03%) comparable to L-ascorbic acid. The extract showed significant cytotoxicity ($p < 0.05$) against HeLa cells while exhibiting minimal toxicity to Peripheral Blood Mononuclear Cells (PBMCs).

At a concentration of 25 µg/mL, the methanolic extract notably inhibited proliferation of MDA-MB-231 cells more effectively than MCF-7 cells. Adhesion and migration assays demonstrated reduced metastatic potential, while DAPI staining confirmed decreased cell adhesion. Flow cytometric analysis revealed apoptosis induction and cell cycle arrest at the G0/G1 phase in MCF-7 and the G2/M phase in MDA-MB-231 cells. Caspase-3 immunoblotting verified apoptosis through caspase-dependent pathways. GC-MS analysis identified benzyl isocyanate as a major bioactive compound may responsible for inhibiting the Slug transcription factor and thereby enhancing E-cadherin expression, thus suppressing EMT.

Overall, *Moringa oleifera* methanolic leaf extract exhibits potent antioxidant, cytotoxic, and anti-metastatic activities. The results suggest that benzyl isocyanate plays a central role in mediating apoptosis and EMT inhibition, positioning *Moringa oleifera* as a promising, low-cost phyto-therapeutic agent for preventing breast cancer metastasis.

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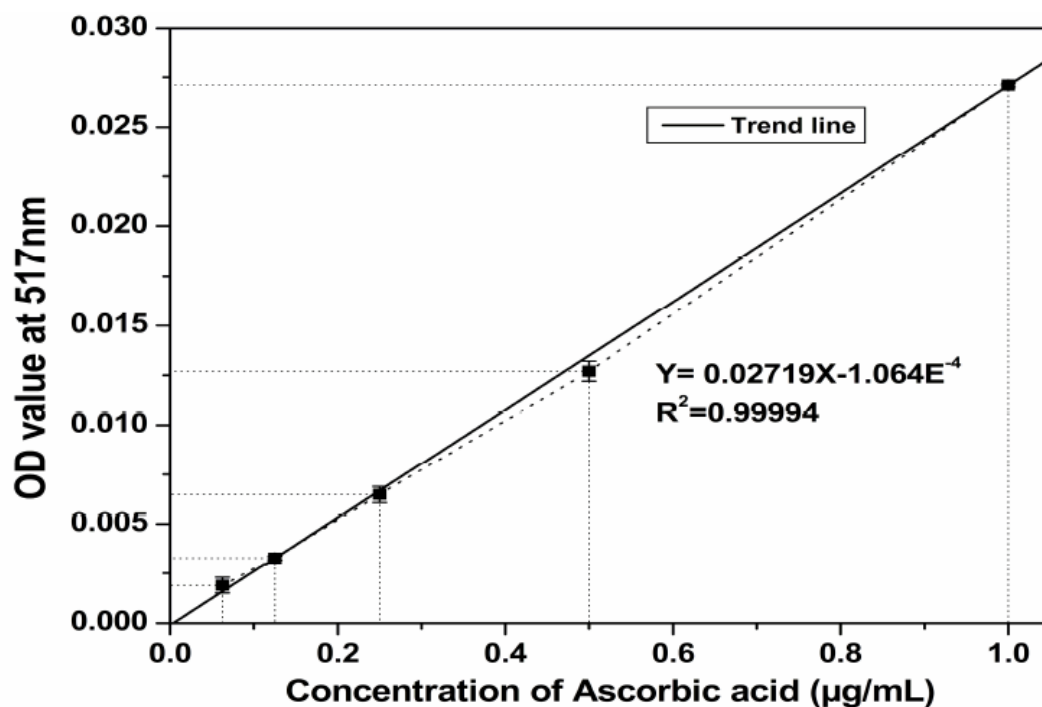


Figure S1: Standard Curve for Ascorbic Acid.

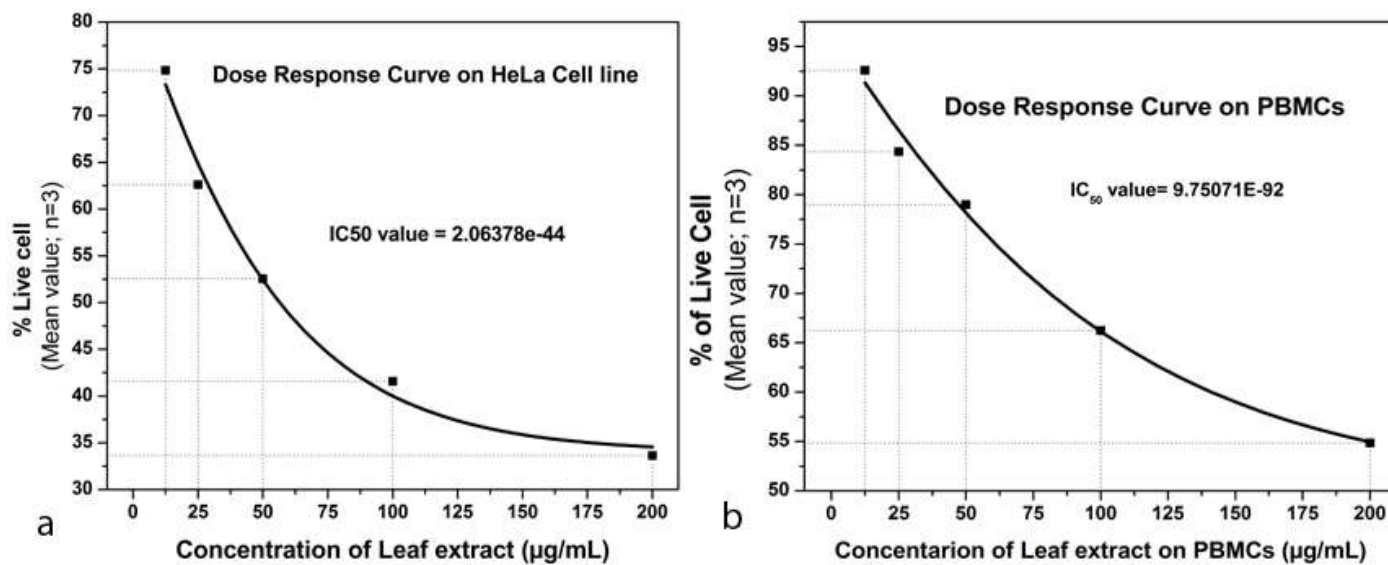


Figure S2: Dose Response Curve for calculation of IC_{50} Value of the leaf extract on HeLa cell line and PBMCs.

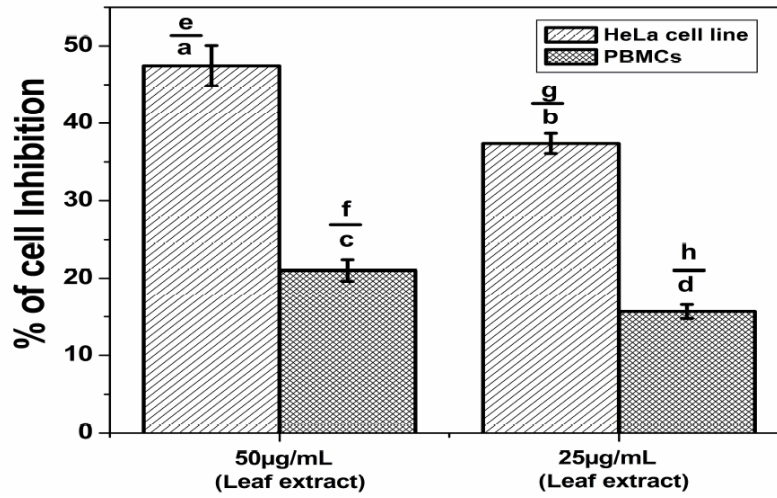


Figure S3: Dose selection between 50 µg/mL and 25 µg/mL.

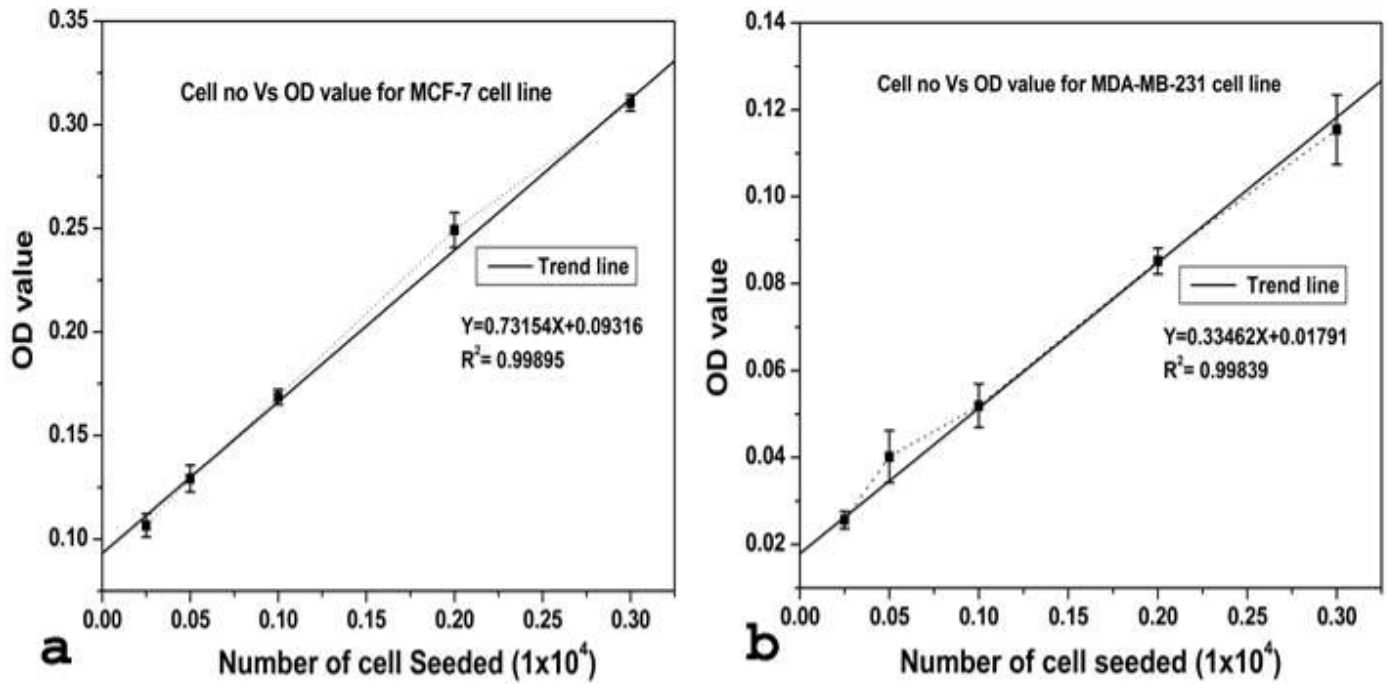


Figure S4: Regression data analysis of cell vs OD for MCF-7 and MDA-MB-231 cell line.