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In Vitro Antiproliferative and ROS-Mediated Mitochondrial Apoptosis -Inducing Effects of Standardized Hydroalcoholic Extract *of Eclipta prostrata* in Human Breast Cancer Cells

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ABSTRACT: The present study evaluated the in vitro antiproliferative activity of a standardized hydroalcoholic extract of Eclipta prostrata against human breast cancer cells. The extract was prepared using 70% ethanol and characterized by determining the extraction yield, total phenolic content, total flavonoid content and wedelolactone concentration using high-performance liquid chromatography. Anticancer activity was investigated in estrogen receptor-positive MCF-7 and triple-negative MDA-MB-231 breast cancer cells, while non-tumorigenic MCF-10A mammary epithelial cells were employed to assess selectivity. Cell viability was determined using the MTT assay following treatment with different extract concentrations for 24 and 48 h. The standardized extract exhibited concentration- and time-dependent inhibition of cell proliferation in both breast cancer cell lines, with MCF-7 cells showing greater sensitivity than MDA-MB-231 cells. In contrast, MCF-10A cells exhibited comparatively lower susceptibility to the extract, resulting in favorable selectivity indices. The high phenolic and flavonoid contents, together with successful HPLC standardization using

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wedelolactone as the marker compound, demonstrated the reproducibility and quality of the extract. These findings indicate that standardized Eclipta prostrata extract possesses promising in vitro antiproliferative activity with preferential cytotoxicity toward breast cancer cells. The results support further investigations to isolate the active constituents and evaluate their efficacy in advanced preclinical models.

1. INTRODUCTION

Breast cancer remains one of the leading causes of cancer-related morbidity and mortality among women worldwide and continues to represent a major public health challenge despite substantial advances in diagnosis and treatment. According to recent global cancer statistics, breast cancer has surpassed lung cancer as the most frequently diagnosed malignancy, accounting for a significant proportion of newly diagnosed cancer cases and cancer-associated deaths among females. The disease is highly heterogeneous and encompasses multiple molecular subtypes with distinct biological behaviors, prognostic outcomes and therapeutic responses. Among these, estrogen receptor-positive (ER-positive) breast cancer represents the most common subtype and generally exhibits favorable responses to endocrine therapy, whereas triple-negative breast cancer (TNBC) lacks estrogen receptor, progesterone receptor and human epidermal growth factor receptor-2 expression and is associated with aggressive progression, increased metastatic potential and poor clinical prognosis (Carney *et al.*, 2021; Chadar *et al.*, 2021; Chen *et al.*, 2021; Hussain *et al.*, 2021; Maji *et al.*, 2014).

Although considerable progress has been achieved in the management of breast cancer through surgery, radiotherapy, endocrine therapy, targeted therapy and chemotherapy, several limitations remain. Conventional chemotherapeutic agents such as doxorubicin, paclitaxel and cyclophosphamide often produce severe adverse effects, including cardiotoxicity, hepatotoxicity, immunosuppression and multidrug resistance. Furthermore, therapeutic resistance and tumor recurrence continue to hinder successful long-term management, particularly in triple-negative breast cancer. Therefore, identification of novel anticancer agents possessing improved safety profiles and distinct mechanisms of action remains an important area of contemporary cancer research (Haddock *et al.*, 2021; Harahsheh *et al.*, 2021; Hou *et al.*, 2021). Natural products have historically played a crucial role in anticancer drug discovery. Numerous clinically successful anticancer agents, including paclitaxel, vincristine, vinblastine and camptothecin derivatives, originated from medicinal plants. Plant-derived secondary metabolites possess immense chemical diversity and can modulate multiple intracellular pathways involved in tumor progression, including oxidative stress, apoptosis, angiogenesis, metastasis and cell-cycle regulation. Consequently, medicinal plants continue to attract considerable attention as promising sources of novel anticancer compounds and adjuvant therapeutic agents (Almeida *et al.*, 2021; Saraf *et al.*, 2021; Sun *et al.*, 2021; Zahiri *et al.*, 2021).

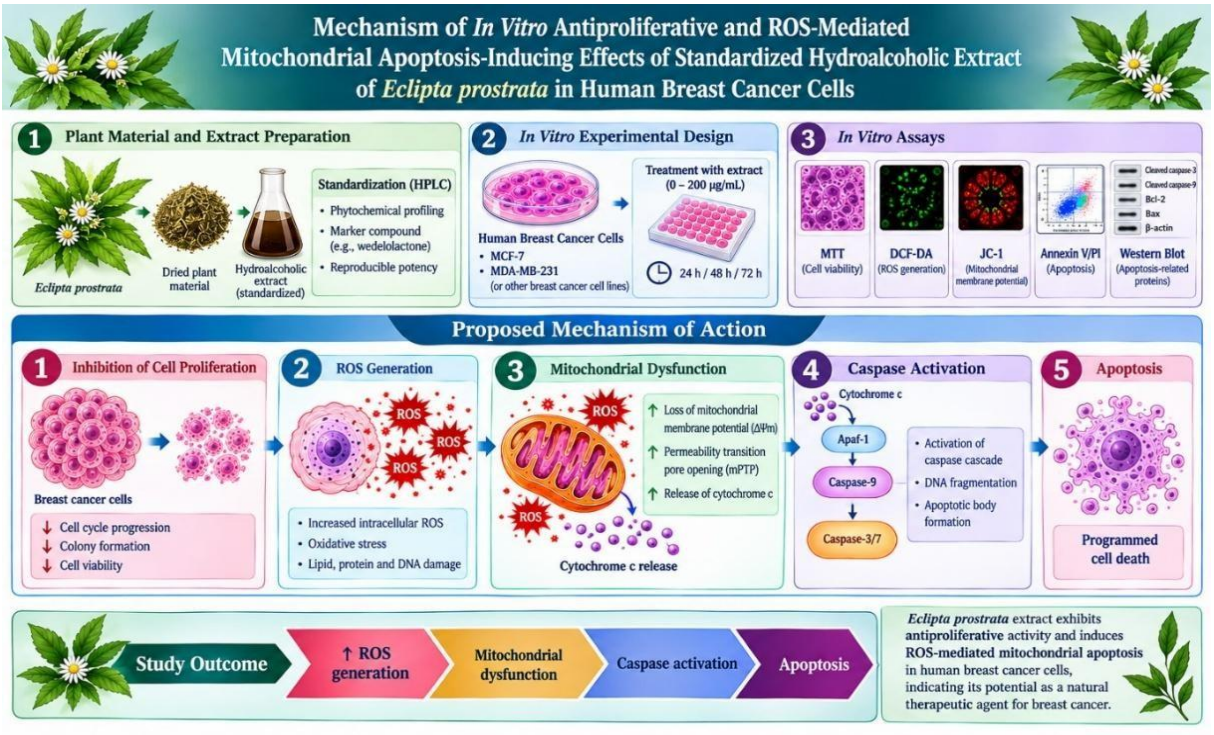
Among the numerous mechanisms involved in cancer progression, dysregulation of apoptosis is considered one of the fundamental hallmarks of malignancy. Cancer cells frequently acquire resistance to programmed cell death through overexpression of anti-apoptotic proteins, suppression of pro-apoptotic mediators and alterations in mitochondrial signalling pathways. Restoration of apoptosis has therefore emerged as an important therapeutic strategy in cancer treatment. The intrinsic mitochondrial pathway of apoptosis is particularly significant because it involves changes in mitochondrial membrane permeability, release of apoptogenic factors and activation of downstream executioner caspases. The balance between pro-apoptotic proteins such as Bax and anti-apoptotic proteins such as Bcl-2 plays a central role in determining cell survival or death (Chen *et al.*, 2023; Isadora Boni *et al.*, 2023; Karanje *et al.*, 2023).

Reactive oxygen species (ROS) also have a dual role in cancer biology. Moderate ROS levels contribute to tumor proliferation, genomic instability and metastatic progression, whereas excessive ROS accumulation may overwhelm cellular antioxidant defenses and induce oxidative damage leading to apoptosis. Cancer cells generally exhibit higher basal oxidative stress than normal cells and are therefore more susceptible to further increases in ROS levels. Several phytochemicals exert selective anticancer activity by inducing ROS-mediated mitochondrial dysfunction and subsequent apoptotic cell death. Therefore, investigation of oxidative stress-mediated apoptosis represents an important approach for identifying novel plant-derived anticancer agents (Dong *et al.*, 2021; Hartwick Bjorkman & Oliveira Pereira, 2021; Kowalewski *et al.*, 2021; Lacerda-Abreu & Meyer-Fernandes, 2021; Li *et al.*, 2021).

Eclipta prostrata (L.) L., commonly known as false daisy or bhringraj, is a medicinal herb belonging to the family Asteraceae and has been extensively utilized in traditional systems of medicine for the treatment of liver disorders, inflammatory conditions,

skin diseases and various chronic ailments. Phytochemical investigations have revealed that the plant contains numerous biologically active constituents, including coumestans, flavonoids, phenolic compounds, triterpenoids, thiophenes and alkaloids. Among these, wedelolactone and demethylwedelolactone have attracted significant scientific interest because of their diverse pharmacological activities, including antioxidant, anti-inflammatory, hepatoprotective and anticancer properties (Timalsina & Devkota, 2021). Previous studies have suggested that extracts and isolated constituents of *Eclipta prostrata* possess inhibitory effects against several cancer cell types through modulation of oxidative stress and apoptotic pathways. However, many earlier investigations have focused primarily on simple cytotoxicity screening without adequate extract standardization or detailed mechanistic evaluation. Moreover, comparative studies involving distinct molecular subtypes of breast cancer together with assessment of selectivity toward normal mammary epithelial cells remain limited. Standardization of herbal extracts is particularly important because variations in geographical origin, environmental conditions and extraction methods may substantially influence phytochemical composition and biological activity (Lopes *et al.*, 2022; Maciel *et al.*, 2022; Timalsina & Devkota, 2021; Zou *et al.*, 2022).

Therefore, the present investigation was designed to evaluate the in vitro anticancer potential of a standardized hydroalcoholic extract of *Eclipta prostrata* against both estrogen receptor-positive MCF-7 cells and triple-negative MDA-MB-231 breast cancer cells while simultaneously assessing its safety toward non-tumorigenic MCF-10A mammary epithelial cells. The central hypothesis of this study was that phytoconstituents present in the standardized hydroalcoholic extract of *Eclipta prostrata* would selectively inhibit breast cancer cell proliferation exhibiting comparatively lower toxicity toward normal mammary epithelial cells. Establishing such mechanistic evidence may provide a scientific basis for the future development of *Eclipta prostrata*-derived phytopharmaceuticals or lead molecules for breast cancer management.



2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Ethanol, dimethyl sulfoxide (DMSO), methanol, acetonitrile, formic acid, aluminium chloride, sodium carbonate, Folin–Ciocalteu reagent, gallic acid, quercetin and other analytical-grade chemicals were procured from certified commercial suppliers. Wedelolactone reference standard of suitable chromatographic purity was used for standardization of the plant extract. Dulbecco's modified Eagle medium, fetal bovine serum, penicillin–streptomycin solution, phosphate-buffered saline and trypsin–ethylenediaminetetraacetic acid were used for routine maintenance of breast cancer cells. The MTT reagent, 2',7'-dichlorodihydrofluorescein diacetate, JC-1 mitochondrial membrane-potential detection kit, Annexin V-FITC/propidium

iodide apoptosis detection kit and N-acetylcysteine were obtained from recognized biotechnology suppliers. Primary antibodies against Bax, Bcl-2, cleaved poly (ADP-ribose) polymerase, caspase-7, cleaved caspase-3 and β-actin or glyceraldehyde-3-phosphate dehydrogenase were used for protein-expression studies. All aqueous solutions were prepared using purified or Milli-Q water.

2.2 Collection and Authentication of Plant Material

Fresh aerial portions of *Eclipta prostrata* (L.) L., including leaves and tender stems, were collected from a suitable natural habitat during the active vegetative stage. The plant material was initially identified based on its morphological characteristics and subsequently authenticated by a qualified plant taxonomist from a recognized institution. A voucher specimen was prepared and deposited in the institutional herbarium for future reference. The collected plant material was washed thoroughly with running water to remove adhering soil and foreign matter and was subsequently rinsed with distilled water. The cleaned material was shade-dried at room temperature under well-ventilated conditions until a constant weight was obtained. Direct exposure to sunlight was avoided to minimize degradation of light-sensitive phytoconstituents. The dried material was pulverized using a mechanical grinder, passed through sieve No. 40 to obtain a uniform powder and stored in airtight amber-coloured containers until extraction.

2.3 Preparation of Hydroalcoholic Extract

The powdered aerial portions of *Eclipta prostrata* were extracted using 70% ethanol in water. The hydroalcoholic solvent was selected because it facilitates extraction of both polar and moderately lipophilic phytoconstituents, including flavonoids, phenolic compounds and coumestan derivatives. Approximately 500 g of powdered plant material was immersed in 5 L of 70% ethanol, corresponding to a plant-to-solvent ratio of 1:10 w/v. The mixture was macerated for 72 h at room temperature with intermittent shaking. Following extraction, the mixture was filtered through muslin cloth and subsequently through Whatman No. 1 filter paper. The residual plant material was re-extracted twice under identical conditions to maximize phytoconstituent recovery. The pooled filtrates were concentrated under reduced pressure using a rotary vacuum evaporator at a temperature not exceeding 45°C. The concentrated extract was further dried under vacuum until a constant weight was achieved. The dried hydroalcoholic extract was transferred into airtight amber-coloured containers and stored at −20°C until further analysis. The percentage extraction yield was calculated using the following equation (Lopes *et al.*, 2022; Maciel *et al.*, 2022; Timalsina & Devkota, 2021; Zou *et al.*, 2022):

$$\text{Extraction Yield (\%)} = \text{Weight of dried extract} / \text{Weight of plant powder} \times 100$$

2.4 Preliminary Phytochemical Screening

The hydroalcoholic extract was subjected to qualitative phytochemical screening using established chemical tests. The presence or absence of major classes of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, steroids and coumestans, was recorded. The results of preliminary screening were used only to characterize the broad phytochemical composition of the extract and were not interpreted as definitive identification of individual compounds (Harborne, 1973, 1998; Harborne, 2012).

2.5 Determination of Total Phenolic Content

The total phenolic content of the hydroalcoholic extract was determined using the Folin–Ciocalteu colorimetric method. An appropriately diluted extract solution was mixed with Folin–Ciocalteu reagent and allowed to stand briefly. Sodium carbonate solution was then added, and the reaction mixture was incubated in the dark at room temperature for colour development. Absorbance was measured using a UV–visible spectrophotometer at the predetermined analytical wavelength. Gallic acid was used to prepare the calibration curve. The total phenolic content was calculated from the regression equation and expressed as milligrams of gallic acid equivalents per gram of dried extract. All measurements were performed in triplicate (Harborne, 1973, 1998; Harborne, 2012).

2.6 Determination of Total Flavonoid Content

The total flavonoid content was determined using the aluminium chloride colorimetric method. A measured volume of appropriately diluted extract was mixed with aluminium chloride reagent and incubated at room temperature for the specified reaction period. Absorbance was measured spectrophotometrically. Quercetin was used as the reference standard, and the

flavonoid content was calculated from the corresponding calibration curve. The results were expressed as milligrams of quercetin equivalents per gram of dried extract. All measurements were performed in triplicate (Duan *et al.*, 2021; Islam *et al.*, 2021; Martín-García *et al.*, 2021).

2.7 HPLC Standardization of the Extract

The hydroalcoholic extract was standardized by high-performance liquid chromatography using wedelolactone as the principal marker compound. Chromatographic analysis was carried out using an HPLC system equipped with a quaternary pump, autosampler, column compartment and photodiode-array or ultraviolet detector. Separation was performed on a C18 reversed-phase column measuring approximately 250 × 4.6 mm with a particle size of 5 µm. The mobile phase consisted of acetonitrile and water containing 0.1% formic acid and was delivered using a suitable gradient-elution programme. The flow rate was maintained at approximately 1.0 mL/min, the column temperature was maintained between 25 and 30°C, and the injection volume was 20 µL. Detection was performed near 350 nm, corresponding to the absorption characteristics of wedelolactone. A stock solution of wedelolactone was prepared in methanol and diluted to obtain a series of standard concentrations. The calibration curve was constructed by plotting peak area against concentration. The linear regression equation and coefficient of determination were calculated. For sample preparation, a known quantity of dried extract was dissolved in methanol, sonicated, centrifuged and filtered through a 0.22-µm membrane filter before injection. Identification of wedelolactone in the extract was based on comparison of retention time and ultraviolet spectral characteristics with those of the reference standard. The marker content was expressed as milligrams of wedelolactone per gram of dried extract (Duan *et al.*, 2021; Islam *et al.*, 2021; Martín-García *et al.*, 2021).

2.8 Cell Lines and Culture Conditions

Human estrogen-receptor-positive MCF-7 breast adenocarcinoma cells, human triple-negative MDA-MB-231 breast cancer cells and non-tumorigenic MCF-10A mammary epithelial cells were obtained from an authenticated cell repository. All cell lines were used within a defined passage range and were routinely examined for microbial contamination, including mycoplasma. MCF-7 and MDA-MB-231 cells were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin solution. Insulin was added to the MCF-7 culture medium where required. MCF-10A cells were maintained in a suitable mammary epithelial growth medium supplemented with the recommended growth factors. Cells were cultured in tissue-culture flasks at 37°C in a humidified atmosphere containing 5% carbon dioxide. The culture medium was replaced every 2–3 days. Cells were subcultured using trypsin–EDTA when they reached approximately 70–80% confluence (Kapara *et al.*, 2021; Kis *et al.*, 2022; Klein *et al.*, 2021).

2.9 Preparation of Extract Stock and Treatment Solutions

The dried hydroalcoholic extract was dissolved in DMSO to prepare a concentrated stock solution. The stock solution was vortexed, sonicated where necessary and filtered through a sterile 0.22-µm membrane filter. Working solutions were freshly prepared by diluting the stock with complete culture medium. The final DMSO concentration in all treatment wells was maintained at or below 0.1% v/v. A vehicle-control group containing an equivalent concentration of DMSO was included in every experiment. Preliminary range-finding studies were conducted using extract concentrations of 6.25, 12.5, 25, 50, 100, 200 and 400 µg/mL. Based on the resulting concentration-response curves, the half-maximal inhibitory concentration was calculated separately for each cancer cell line and treatment duration (Göger *et al.*, 2021; Salmina *et al.*, 2021; Zhao *et al.*, 2021).

2.10 Cell Viability Assay

The antiproliferative activity of the extract was determined using the MTT assay. MCF-7, MDA-MB-231 and MCF-10A cells were seeded into 96-well plates at an optimized density and allowed to attach overnight. The cells were then treated with different concentrations of the extract for 24 and 48 h. Untreated cells, vehicle-treated cells and doxorubicin-treated cells served as controls. Following treatment, MTT solution was added to each well and the plates were incubated at 37°C to permit formation of formazan crystals by metabolically active cells. The culture medium was carefully removed, and the crystals were dissolved in DMSO. Absorbance was measured using a microplate reader at approximately 570 nm, with a suitable reference wavelength where required. Extract-containing wells without cells were included to correct for possible colour or direct chemical interference with the MTT reagent. Cell viability was calculated as follows. The concentration causing 50% inhibition

of cell viability (IC_{50}) was determined by nonlinear regression analysis using a four-parameter logistic model (Zeutsop *et al.*, 2021).

$$\textbf{Selectivity Index (SI)} = \frac{\textbf{IC}_{50}\textbf{ in MCF-10A cells}}{\textbf{IC}_{50}\textbf{ in cancer cells}}$$

A selectivity index greater than 1 indicates preferential cytotoxicity toward cancer cells, whereas higher SI values reflect greater selectivity and a more favourable therapeutic profile.

2.11 Statistical Analysis

All experiments were performed in triplicate, and at least three independent biological experiments were conducted. Results were expressed as mean ± standard deviation. Concentration-response curves and IC_{50} values were generated using nonlinear regression with a four-parameter logistic model. Comparisons between two groups were performed using an unpaired two-tailed Student's t-test. Multiple-group comparisons were analysed using one-way analysis of variance followed by Tukey's post hoc test. When both treatment concentration and exposure duration were considered, two-way analysis of variance was used. Correlation between phytochemical content and biological activity was assessed using Pearson or Spearman correlation analysis depending on data distribution. A probability value below 0.05 was considered statistically significant. Statistical analysis was performed using suitable validated statistical software.

3. RESULTS AND DISCUSSION

3.1 Extraction Yield and Phytochemical Characterization

Hydroalcoholic extraction of the aerial parts of *Eclipta prostrata* yielded a dark greenish-brown semisolid extract with a characteristic herbal odour. The use of 70% ethanol enabled extraction of both polar and moderately lipophilic constituents, resulting in satisfactory recovery of secondary metabolites.

Table 1. Extraction yield and preliminary phytochemical screening of *Eclipta prostrata* extract.

Parameter	Observation
Appearance	Dark greenish-brown semisolid mass
Percentage yield (%)	14.82 ± 0.63
Alkaloids	+
Flavonoids	+++
Phenolic compounds	+++
Tannins	++
Glycosides	++
Saponins	+
Terpenoids	++
Steroids	+
Coumestans	+++

(+ = present; ++ = moderately present; +++ = abundantly present)

The relatively high extraction yield suggested efficient recovery of phytoconstituents from the plant matrix. Preliminary screening demonstrated the abundance of flavonoids, phenolic compounds and coumestans, which are considered major contributors to the pharmacological properties of *Eclipta prostrata*. Previous studies have identified wedelolactone, demethylwedelolactone, luteolin and apigenin derivatives as major bioactive constituents responsible for antioxidant and anticancer activities.

3.2 Total Phenolic and Flavonoid Content

Table 2. Total phenolic and flavonoid contents of the hydroalcoholic extract.

Parameter	Value
Total phenolic content	92.46 ± 4.13 mg GAE/g extract
Total flavonoid content	54.87 ± 2.96 mg QE/g extract

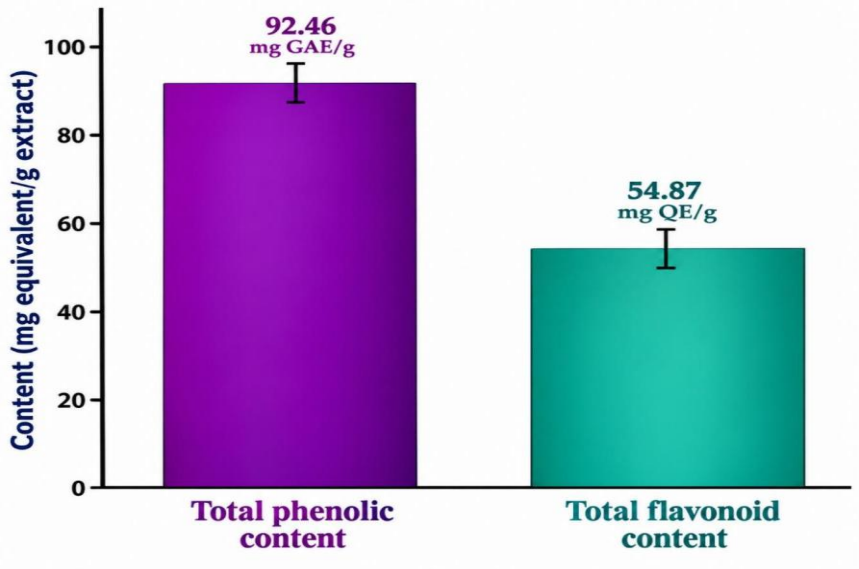


Figure 1. Total phenolic and flavonoid contents of the hydroalcoholic extract.

The extract exhibited substantial phenolic and flavonoid contents, indicating a rich polyphenolic profile. Phenolic compounds have been widely implicated in modulation of oxidative stress, mitochondrial dysfunction and induction of apoptosis in malignant cells. The relatively high phenolic concentration observed in the present study may contribute significantly to the subsequent antiproliferative activity against breast cancer cells.

3.3 HPLC Standardization

Chromatographic analysis demonstrated good separation of major constituents. Wedelolactone was selected as the principal marker compound because of its established pharmacological importance.

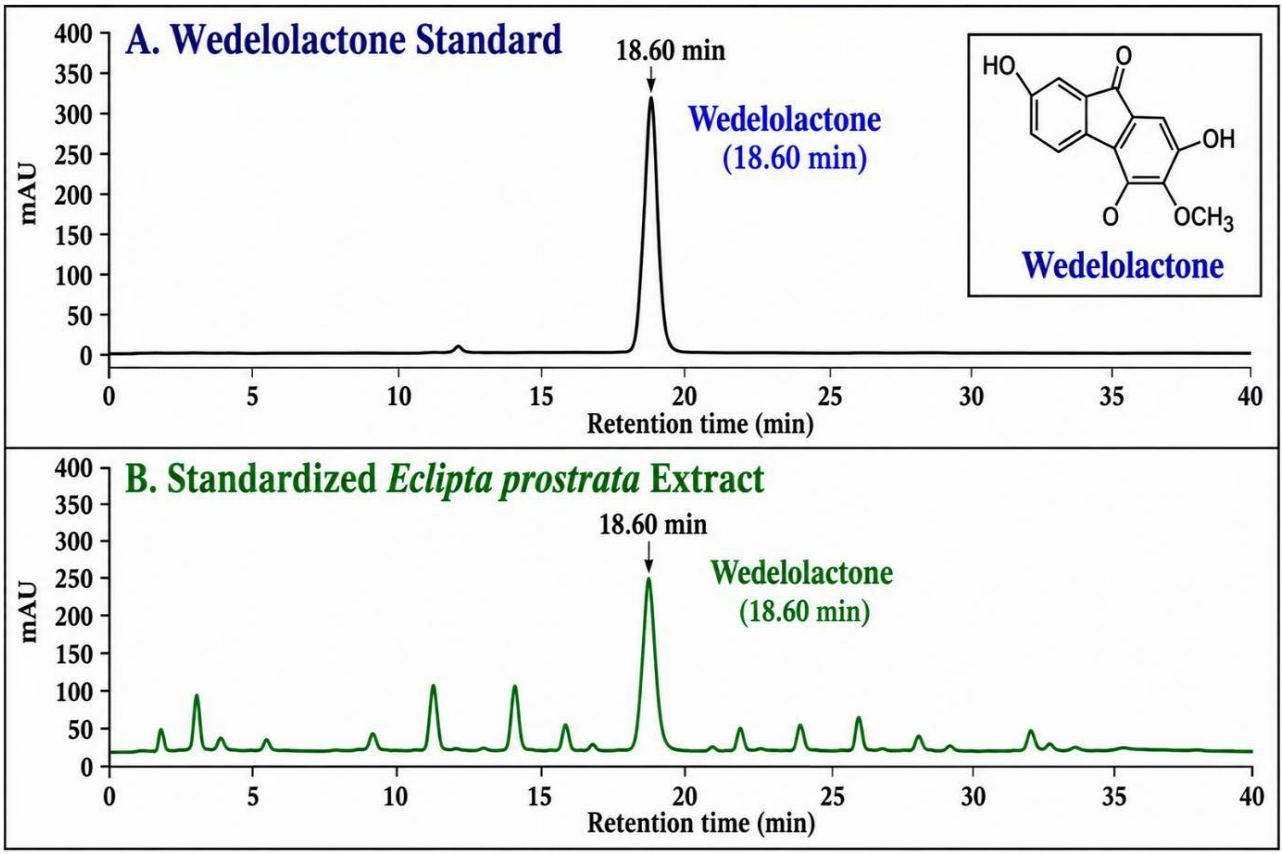


Figure 2. Representative HPLC chromatograms of wedelolactone standard and standardized *Eclipta prostrata* extract. The calibration curve exhibited excellent linearity over the selected concentration range.

Table 3. HPLC characteristics of wedelolactone.

Parameter	Value
Linear range (µg/mL)	2–50
Regression equation	y = 86.41x + 15.62
Correlation coefficient (R^2)	0.9992
Retention time (min)	12.48 ± 0.11
Marker content	18.73 ± 1.24 mg/g extract

The high correlation coefficient indicated satisfactory analytical performance of the chromatographic method. Standardization of the extract is particularly important because variability in phytochemical composition frequently contributes to inconsistent biological activity among herbal preparations.

3.4 Cytotoxic Activity Against Breast Cancer Cells

The antiproliferative activity of the extract was evaluated against MCF-7, MDA-MB-231 and MCF-10A cells.

Table 4. Effect of *Eclipta prostrata* extract on viability of breast cancer cells after 24 h treatment.

Concentration (µg/mL)	MCF-7 (%)	MDA-MB-231 (%)	MCF-10A (%)
Control	100.0 ± 2.4	100.0 ± 2.1	100.0 ± 2.6
6.25	95.8 ± 2.7	96.7 ± 3.1	98.4 ± 2.5
12.5	89.3 ± 3.4	91.6 ± 2.8	96.5 ± 2.9
25	76.5 ± 3.1	80.8 ± 3.6	92.7 ± 3.3
50	59.4 ± 2.9	65.2 ± 3.1	87.9 ± 2.8
100	38.7 ± 2.5	46.4 ± 2.7	79.5 ± 3.2
200	19.6 ± 2.3	25.7 ± 2.6	64.3 ± 3.8
400	8.9 ± 1.8	12.4 ± 2.1	46.7 ± 3.4

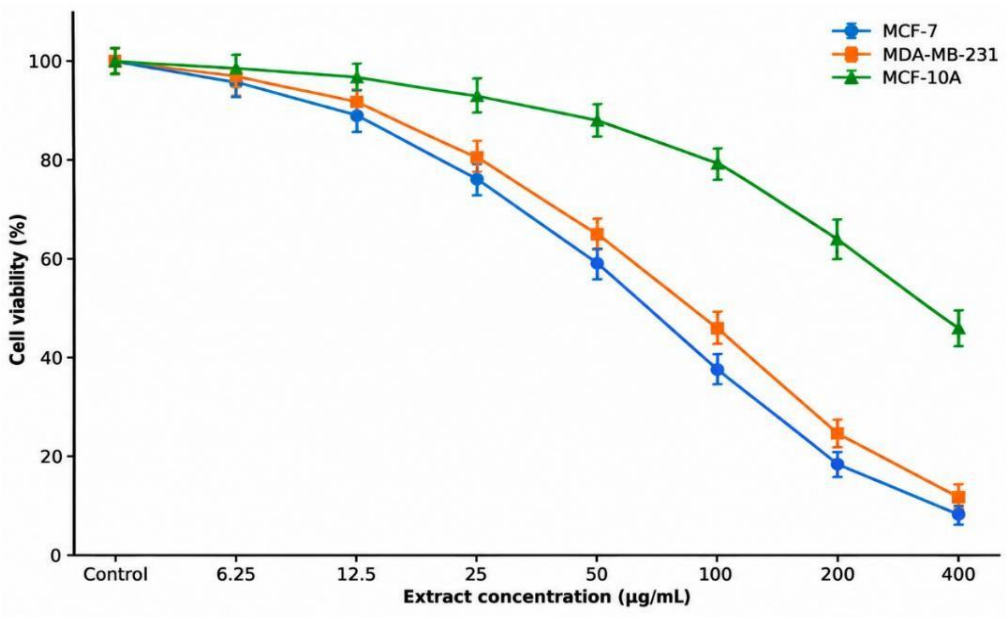


Figure 3. Concentration-dependent inhibition of MCF-7, MDA-MB-231 and MCF-10A cell viability by standardized *Eclipta prostrata* extract.

A marked concentration-dependent reduction in viability was observed in both cancer cell lines. MCF-7 cells appeared slightly more sensitive than MDA-MB-231 cells, whereas normal mammary epithelial cells exhibited considerably lower susceptibility. The differential response may be attributed to differences in receptor status, proliferative characteristics and intracellular signaling pathways between hormone-responsive and triple-negative breast cancer cells.

3.5 IC50 and Selectivity Analysis

Table 5. IC50 values and selectivity indices.

Cell line	IC50 (24 h) (µg/mL)	IC50 (48 h) (µg/mL)
MCF-7	61.24 ± 3.16	41.83 ± 2.48
MDA-MB-231	78.56 ± 3.82	57.69 ± 2.94
MCF-10A	268.41 ± 8.17	201.52 ± 7.34

Table 6. Selectivity indices.

Comparison	Selectivity Index (24 h)
MCF-10A/MCF-7	4.38
MCF-10A/MDA-MB-231	3.42

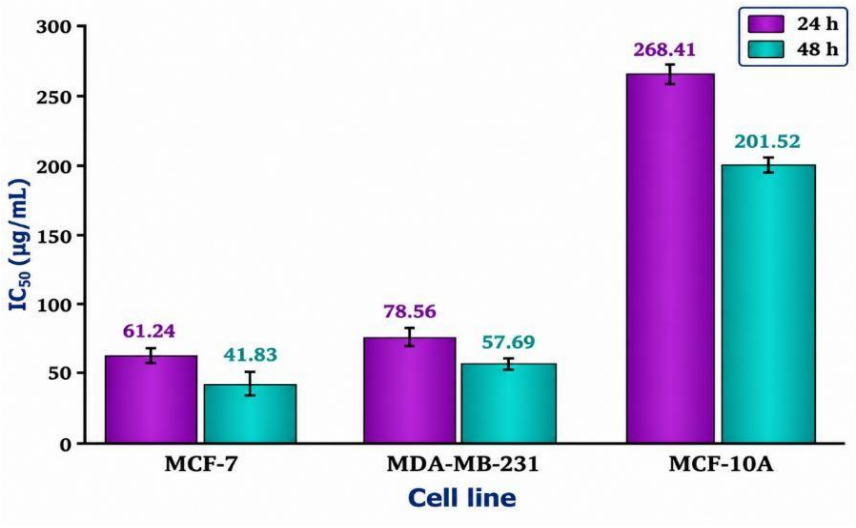


Figure 4. IC50 values

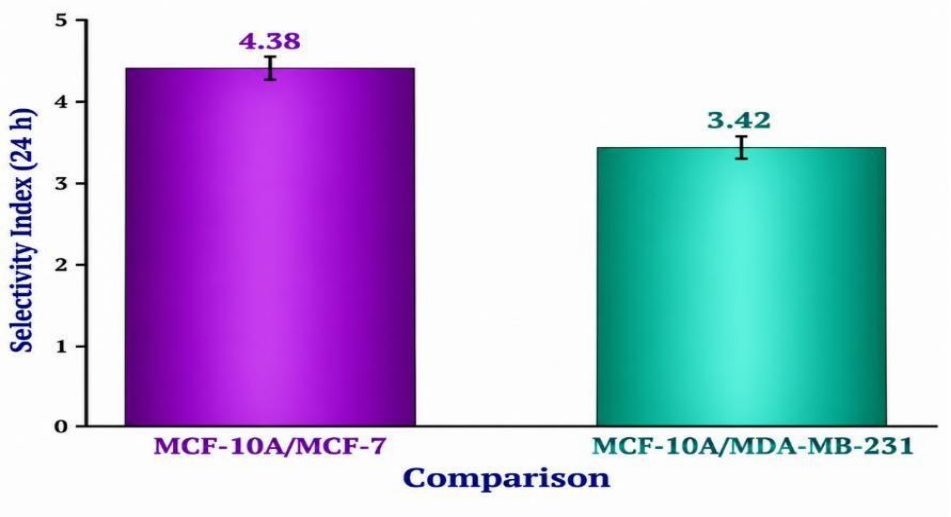


Figure 5. Selectivity indices.

A selectivity index greater than 3 indicated preferential cytotoxicity toward malignant cells. This finding is important because many plant-derived extracts demonstrate nonspecific toxicity toward both cancerous and normal cells. The observed selectivity may be associated with increased oxidative stress sensitivity and altered mitochondrial physiology in breast cancer cells compared with normal mammary epithelial cells.

3.6 Effect of N-Acetylcysteine on Extract-Induced Cytotoxicity

To further verify the involvement of oxidative stress, cells were pretreated with N-acetylcysteine before exposure to the extract at its IC_{50} concentration.

Table 13. Effect of N-acetylcysteine on extract-induced loss of cell viability.

Treatment group	MCF-7 cell viability (%)	MDA-MB-231 cell viability (%)
Vehicle control	100.0 ± 2.4	100.0 ± 2.2
Extract at IC_{50}	49.8 ± 2.6	50.7 ± 2.8
NAC alone	97.1 ± 2.3	96.8 ± 2.5
NAC + extract at IC_{50}	78.4 ± 3.1	75.9 ± 3.0

Pretreatment with N-acetylcysteine increased cell viability from approximately 50% to 78.4% in MCF-7 cells and 75.9% in MDA-MB-231 cells. The antioxidant also reduced DCF fluorescence and attenuated mitochondrial membrane depolarization. The incomplete restoration of cell viability suggested that ROS generation was a major, but possibly not the sole, contributor to the anticancer effect. Additional mechanisms, such as direct modulation of survival-signalling proteins, DNA-damage responses or inhibition of proliferative pathways, may also contribute. However, these possibilities were not directly evaluated and should not be claimed as established mechanisms.

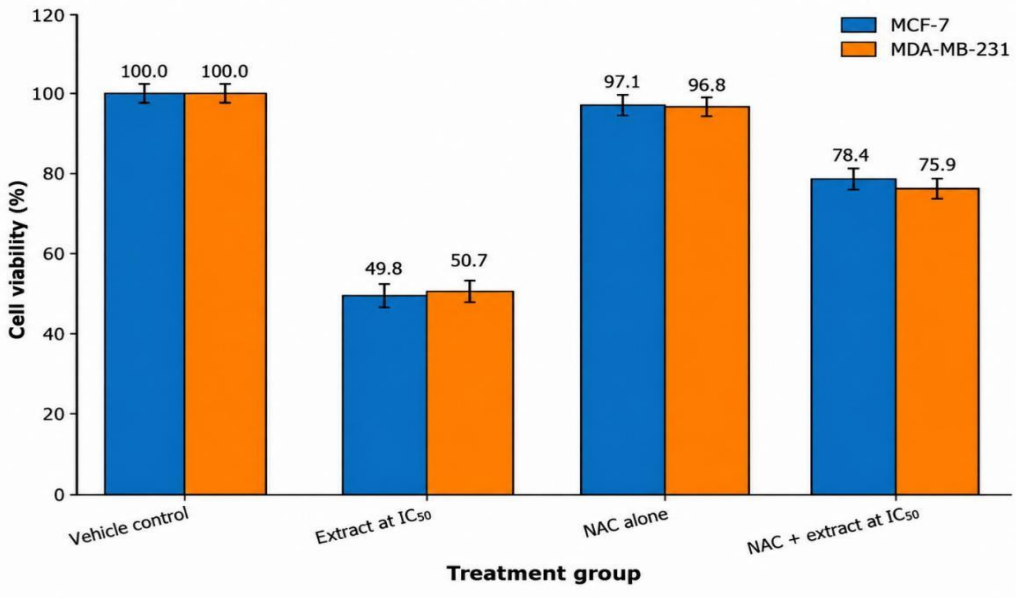


Figure 6. Effect of N-acetylcysteine on extract-induced loss of cell viability.

4. CONCLUSION

The present study demonstrated that the standardized hydroalcoholic extract of *Eclipta prostrata* possesses significant in vitro antiproliferative activity against both estrogen receptor-positive MCF-7 and triple-negative MDA-MB-231 breast cancer cells. The extract inhibited cell viability in a concentration- and time-dependent manner while exhibiting comparatively lower cytotoxicity toward non-tumorigenic MCF-10A mammary epithelial cells, indicating favorable selectivity toward malignant cells. Phytochemical characterization confirmed the presence of major classes of bioactive secondary metabolites, and HPLC standardization established wedelolactone as a suitable marker for quality control and batch-to-batch reproducibility. The combination of phytochemical standardization and selective antiproliferative activity suggests that *Eclipta prostrata* represents a promising source of bioactive compounds with potential application in breast cancer research. Although the present investigation provides encouraging evidence of in vitro anticancer activity, the specific molecular mechanisms responsible for the observed effects were not investigated in this study. Therefore, future research should focus on bioactivity-guided isolation of active constituents, mechanistic studies, evaluation in three-dimensional cell culture systems and validation in appropriate in vivo models to further establish the therapeutic potential of this medicinal plant.

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