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Phytochemical characterization, antioxidant activity and *In -Vitro* cytotoxic effects of *Heliotropium marifolium* (J. Koenig ex Retz.) Leaf Extract against MCF-7 breast cancer cells

Nithya S[1*], Wesely Jebasing Devairakkam E G[2], Thangadurai K[3]
[1] Scholar, Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamilnadu, India
[1] Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamilnadu, India
[2] JSA Medical College for Siddha and Research Centre, Ulundurpet, Tamilnadu, India
Corresponding Author: Nithya S

Abstract
Background: Breast cancer is one of the most frequently diagnosed malignancies among women worldwide, necessitating the exploration of novel therapeutic agents derived from natural products. Medicinal plants containing bioactive phytochemicals have gained considerable interest due to their antioxidant and anticancer properties. This study aimed to investigate the phytochemical profile, antioxidant activity, and cytotoxic potential of the ethanol leaf extract of *Heliotropium marifolium*.
Methods: Qualitative phytochemical screening was performed to identify major secondary metabolites in the plant extract. Antioxidant activity was evaluated using the DPPH free radical Scavenging assay. The cytotoxic effect of the extract was determined using the MTT cell viability assay against MCF-7. Morphological alterations in treated cells were examined by microscopic observation to assess apoptotic features.
Results: Phytochemical analysis confirmed the presence of several biologically active constituents, including alkaloids, flavonoids, tannins, saponins, and terpenoids. The extract demonstrated significant free radical scavenging activity in the DPPH assay, indicating strong antioxidant potential. *In vitro* cytotoxicity assessment revealed a concentration-dependent inhibition of cancer cell proliferation, with an IC_{50} value of 147.8 ± 2.14 µg/mL. Treated cells exhibited characteristic apoptotic morphological changes, including cellular shrinkage, membrane blebbing, and detachment from the culture surface. The reference anticancer agent Doxorubicin showed greater cytotoxic potency with a lower IC_{50} value.
Conclusion: The findings demonstrate that *Heliotropium marifolium* possesses notable antioxidant capacity and moderate antiproliferative activity against breast cancer cells. The observed biological effects may be attributed to the presence of phenolic and flavonoid compounds in the extract. These results highlight the potential of this plant as a promising source of bioactive molecules for the development of novel anticancer therapeutics. Further studies focusing on isolation of active constituents and elucidation of molecular mechanisms are warranted.

Keywords: *Heliotropium marifolium*, Breast cancer, MCF-7 cells, Antioxidant activity, Cytotoxicity, MTT assay, IC_{50}, Apoptosis.

Introduction

Breast cancer is the most commonly diagnosed malignancy among women worldwide and represents a major public health concern due to its high incidence and mortality rates. According to global cancer statistics, breast cancer accounts for a substantial proportion of newly diagnosed cancer cases and cancer-related deaths among women globally. Recent estimates indicate that breast cancer has surpassed lung cancer as the most frequently diagnosed cancer worldwide, highlighting the growing burden of this disease on healthcare systems and society (Sung H, Ferlay J, Siegel RL, *et al.*, 2021.) Although early detection and improved treatment strategies have contributed to better survival outcomes, breast cancer remains a leading cause of cancer mortality in many regions, particularly in low- and middle-income countries.

Current therapeutic approaches for breast cancer include surgery, chemotherapy, radiotherapy, hormone therapy, and targeted therapy. These treatment modalities have significantly improved patient prognosis; however, several limitations still hinder their long-term effectiveness. Chemotherapeutic agents often cause severe side effects due to their non-selective cytotoxicity toward normal cells. Furthermore, the development of drug resistance, tumour recurrence, and metastasis frequently reduces treatment success and contributes to disease progression (Waks AG, Winer EP., 2019) [30]. These challenges highlight the urgent need for safer and more effective therapeutic agents with improved selectivity toward cancer cells.

Natural products derived from medicinal plants have historically played an essential role in drug discovery and development. Many clinically important anticancer drugs have been isolated from plant sources, demonstrating the immense therapeutic potential of natural compounds. Notable examples include paclitaxel, vincristine, vinblastine, and camptothecin derivatives, which have been widely used in cancer chemotherapy (Newman DJ, Crag GM., 2020). Plant-derived bioactive molecules exhibit diverse mechanisms of action, such as inducing apoptosis, suppressing cancer cell proliferation, inhibiting angiogenesis, and modulating oxidative stress pathways. Because of their structural diversity and biological activity, medicinal plants continue to serve as valuable sources for the development of novel anticancer agents (Atanasov AG, Zotchev *et al.*, 2021).

Among medicinal plants, species belonging to the genus *Heliotropium* (family Boraginaceae) have attracted considerable scientific interest due to their diverse pharmacological properties. The genus comprises more than 250 species distributed across tropical and subtropical

regions worldwide. Several *Heliotropium* species have been traditionally used in herbal medicine for the treatment of various ailments, including inflammatory disorders, infections, and skin diseases. Previous pharmacological studies have reported that extracts from different *Heliotropium* species possess antimicrobial, anti-inflammatory, antioxidant, and cytotoxic activities (Rodrigues T, Reker D, *et al*., 2016) [23]. These biological properties are primarily attributed to the presence of various phytochemicals, including flavonoids, alkaloids, phenolic compounds, tannins, and terpenoids.

Phytochemical constituents found in medicinal plants are known to play a crucial role in cancer prevention and therapy. Phenolic compounds and flavonoids, for instance, exhibit strong antioxidant activity and can protect cells from oxidative stress-induced damage, which is often associated with cancer development. Additionally, many plant-derived secondary metabolites have demonstrated the ability to regulate signalling pathways involved in cell cycle progression, apoptosis, and metastasis (Khan H, Saeed M, *et al.,* 2017 [10]). Because oxidative stress and abnormal cell proliferation are closely associated with cancer progression, plant extracts containing these bioactive compounds are increasingly being investigated for their anticancer potential. *Heliotropium marifolium* is a medicinal plant traditionally used in folk medicine for various therapeutic purposes. Preliminary phytochemical investigations have indicated that this species contains a range of biologically active secondary metabolites, including flavonoids, alkaloids, phenolics, tannins, and terpenoids. (Al-Safire., 2018). These compounds are widely recognized for their antioxidant and potential anticancer activities. Despite the growing interest in medicinal plants as sources of anticancer agents, the pharmacological properties of *H. marifolium* have not been extensively explored in scientific studies. In particular, limited research has evaluated its phytochemical composition and potential cytotoxic effects against breast cancer cell lines. (Panche AN, *et al.,* 2016) [19].

Given the increasing demand for novel plant-based therapeutic agents, further investigation into the biological activities of *H. marifolium* is warranted. Therefore, the present study aims to evaluate the phytochemical composition of *Heliotropium marifolium* extract and investigate its antioxidant potential and cytotoxic activity against breast cancer cell lines. The findings of this study may provide valuable insights into the therapeutic potential of this plant and contribute to the identification of novel natural compounds for the development of safer and more effective anticancer therapies.

Materials and Methods

1. Plant Collection and Authentication

Plant samples of *Heliotropium marifolium* were collected from the Mettala hills (Karkudaipatti region) Namakkal District of Tamil Nadu, India. The botanical identity of the plant was verified by taxonomic experts at the Botanical Survey of India (BSI), Coimbatore. A voucher specimen was prepared and deposited in the BSI herbarium for future reference with the assigned voucher number 979.

2. Preparation of Plant Extract

The collected plant materials were carefully cleaned using distilled water to eliminate soil particles and other contaminants. The cleaned samples were then shade-dried under ambient laboratory conditions (25–30 °C) for approximately 10–14 days until the moisture content was completely removed. The dried material was subsequently pulverized using a mechanical grinder to obtain a coarse powder. The powder was sieved to ensure uniform particle size and stored in airtight containers until extraction.

Extraction of the powdered plant material was carried out using the Soxhlet extraction technique with solvents arranged in increasing order of polarity. The extraction process was continued until the solvent in the siphon tube appeared colourless, indicating complete extraction of soluble constituents. The resulting extracts were concentrated under reduced pressure using a rotary evaporator to remove the solvent. The concentrated extracts were then dried and preserved at 4 °C until further experimental use. Based on the results of preliminary screening, the ethanol extract was selected for subsequent phytochemical and biological analyses.

3. Preliminary Phytochemical Screening

The ethanol extract of *Heliotropium marifolium* was subjected to qualitative phytochemical evaluation to determine the presence of major secondary metabolites. Standard phytochemical assays were conducted according to established pharmacognostic procedures to detect different classes of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and phenolic compounds. The presence of these constituents was identified based on characteristic colour development or precipitate formation observed during the chemical reactions.

4. *In vitro* Antioxidant Activity

The antioxidant capacity of the ethanol extracts derived from the leaves and stems of *Heliotropium marifolium* was evaluated using several established *in vitro* free radical scavenging assays. The antioxidant potential of the extracts was assessed against multiple reactive species, including DPPH, ABTS, hydrogen peroxide, superoxide, hydroxyl, and nitric oxide radicals. Different concentrations of the plant extract were examined in each assay to determine their ability to neutralize free radicals. Ascorbic acid was employed as the standard reference antioxidant for comparative evaluation. The percentage inhibition of radicals was calculated for each concentration, and the half maximal inhibitory concentration (IC_{50}) values were determined from the dose–response curves.

5. DPPH Radical Scavenging Activity

The free radical scavenging activity of the extracts against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals was determined using a spectrophotometric method. Various concentrations of the plant extract were mixed with ethanol DPPH solution and allowed to react under dark conditions at room temperature for 30min (Molyneux 2004) [17].

Following incubation, the decrease in absorbance was recorded at 514 nm using a UV–visible spectrophotometer. A reaction mixture without extract served as the control, while ascorbic acid was used as the reference antioxidant. The reduction in absorbance indicated the scavenging of DPPH radicals by the extract. The percentage inhibition was calculated, and the IC_{50} value was obtained from the concentration–inhibition relationship.

6. ABTS Radical Scavenging Activity

The ABTS radical scavenging assay was performed to evaluate the antioxidant capacity of the extracts against ABTS radical cations ($ABTS^{\blacksquare+}$). The ABTS radical solution was prepared by reacting ABTS with potassium persulfate and allowing the mixture to stand in the dark until radical formation occurred. (Re *et al*. 1999). The solution was subsequently diluted to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Different concentrations of the extract were added to the prepared ABTS radical solution and incubated for 10 min under dark conditions. The absorbance of the reaction mixture was measured at 734 nm, and the reduction in absorbance reflected the radical scavenging ability of the extract. Ascorbic acid was used as the positive control, and the percentage inhibition and IC_{50} values were calculated.

2.7 Hydrogen Peroxide Scavenging Activity

The ability of the plant extract to scavenge hydrogen peroxide (H_2O_2) was determined using a spectrophotometric method. Hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4), and different concentrations of the extract were added to the reaction mixture. After a defined incubation period, the absorbance of the mixture was recorded at 230 nm against a blank containing phosphate buffer without hydrogen peroxide. Ascorbic acid served as the standard antioxidant. The reduction in absorbance indicated the capacity of the extract to neutralize hydrogen peroxide radicals. The percentage scavenging activity and IC_{50} values were subsequently calculated.

8. Superoxide Radical Scavenging Activity

Superoxide radical scavenging activity was evaluated using the PMS–NADH–NBT system, which generates superoxide radicals under controlled conditions. The reaction mixture consisted of Tris–HCl buffer, nitro blue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), and phenazine methosulfate (PMS). (Liu *et al.,* 1997) Various concentrations of the plant extract were introduced into the reaction system, and the formation of the colour Formosan product was monitored spectrophotometrically at 560 nm. Ascorbic acid was used as the standard compound. A decrease in absorbance indicated the inhibition of superoxide radical formation. The scavenging percentage and IC_{50} values were determined accordingly.

9. Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was assessed using a Fenton reaction-based assay, which generates hydroxyl radicals through the interaction of hydrogen peroxide with iron ions. The reaction mixture included EDTA–$FeCl_3$, hydrogen peroxide, deoxyribose, phosphate buffer, and various concentrations of the plant extract. (Ruch *et al.* 1989). The mixture was incubated at 37 °C to facilitate radical generation. After incubation, thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were added to develop a colour complex. The absorbance was measured at 532 nm, and ascorbic acid was used as the reference antioxidant. (Halliwell *et al.,* 1996) The inhibition of deoxyribose degradation indicated the hydroxyl radical scavenging capacity of the extract.

10. Nitric Oxide Radical Scavenging Activity

Nitric oxide scavenging activity was determined using a sodium nitroprusside-based assay. Sodium nitroprusside in phosphate buffer generates nitric oxide radicals under physiological conditions, which subsequently react with oxygen to produce nitrite ions. (Govindarajan *et al.*, 2003) [6]. Different concentrations of the plant extract were incubated with sodium nitroprusside solution. After incubation, the reaction mixture was treated with Gris's reagent, which reacts with nitrite ions to form a chromophore measurable at 546 nm. Ascorbic acid served as the reference antioxidant. The reduction in absorbance indicated nitric oxide scavenging activity, and the IC_{50} value was calculated from the inhibition curve.

11. Cytotoxicity Assay (MTT Assay)

11.1 Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), trypsin–EDTA, propidium iodide (PI), and 4',6-diamidino-2-phenylindole (DAPI) were obtained from Sigma-Aldrich and Hi Media Laboratories, India. (Rashid H., *et al.* 2021) [21].

11.2 Cell Culture Maintenance

Human breast cancer cell lines **(**MCF-7) were procured from the National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 µg/mL). The cultures were maintained at 37°C in a humidified incubator with 5% CO_2, ensuring optimal conditions for cell growth and proliferation. (Ali S., *et al.* 2024).

11.3 MTT Cytotoxicity Assay

The cytotoxic potential of the plant extract against breast cancer cells was determined using the MTT colorimetric assay, which measures mitochondrial metabolic activity as an indicator of cell viability. Breast cancer cells (MCF-7) were seeded into 96-well culture plates at a density of 1×10^4 cells per well and incubated overnight to allow proper cell attachment. (Seca A.M.L., Pinto D.C.G.A.2021) [25].Following incubation, the cells were exposed to various concentrations of the plant extract ranging from 50 to 300 µg/mL and further incubated for 24 h under standard culture conditions. After treatment, MTT reagent was added to each well, and the plates were incubated for 4 h to allow viable cells to reduce the MTT reagent into insoluble Formosan crystals. (Choudhari A.S., *et al*. 2021) [4].

The culture medium was subsequently removed, and the resulting Formosan crystals were dissolved using dimethyl sulfoxide (DMSO). The absorbance of the dissolved crystals was measured at 540 nm using a micro plate reader. (Newman D.J., Cragg G.M. (2020). A decrease in

absorbance indicated reduced cell viability and increased cytotoxic activity of the extract. (Choudhari A.S., *et al.* 2021) [4].All experiments were conducted in triplicate to ensure reproducibility. The percentage of viable cells was calculated by comparing the absorbance of treated cells with that of untreated control cells. (Greenwell M., Rahman P.K.S.M. (2020) [7]. The half maximal inhibitory concentration (IC_{50}) value was determined from the dose–response curve to evaluate the anticancer potential of the extract. (Gupta S.C., *et al.* 2020) [8].

11.4 Calculation of Cell Viability

The percentage of cell viability was calculated using the following equation:

Cell viability (%) = (Absorbance of treated cells / Absorbance of control cells) × 100

12. Apoptosis Analysis

The induction of apoptosis in breast cancer cells following treatment with the plant extract was evaluated using propidium iodide (PI) and 4′,6-diamidino-2-phenylindole (DAPI) staining methods. MCF-7 cells were treated with selected concentrations of the extract (150 and 200 µg/mL) and incubated for 24 h under standard culture conditions (Das M., *et al.* 2022) [5]. After treatment, the cells were washed gently with Phosphate-buffered saline (PBS) to remove residual medium and then stained with PI and DAPI according to established staining protocols. The stained cells were subsequently observed under a fluorescence microscope. (Li Y., *et al.* 2022). Apoptotic features such as chromatin condensation, nuclear fragmentation, and loss of membrane integrity were examined. The presence of brightly stained condensed nuclei and fragmented nuclear structures in treated cells was considered indicative of apoptosis when compared with untreated control cells. (Kumari P., *et al.* 2022) [15].

13. Determination of IC_{50} Value

The half maximal inhibitory concentration (IC_{50}) of the plant extract was determined from the cytotoxicity data obtained through the MTT assay. After exposing the cultured cancer cells to different concentrations of the extract, the percentage of viable cells was calculated relative to the untreated control group. (Sharma A., *et al.* 2022) [26]. To estimate the IC_{50} value, the percentage inhibition of cell proliferation was plotted against the corresponding concentrations of the plant extract. The dose–response relationship was analysed using nonlinear regression analysis with Graph Pad Prism software. (Sultana B., *et al.* 2023) [28].

From the generated concentration–response curve, the IC_{50} value was defined as the concentration of the extract required to produce 50% inhibition of cell viability. The analysis was performed using data from three independent experiments, and the mean IC_{50} value was reported. (Alam M.A., *et al.* 2023) [1]. The graphical representation of the dose–response curve was used to illustrate the cytotoxic effect of the extract on the breast cancer cell line.

14. Morphological Observation of Cells

Morphological changes in breast cancer cells after treatment with the plant extract were investigated using phase-contrast microscopy. The human breast cancer cell line (MCF-7) was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. The cells were maintained under standard culture conditions at 37 °C in a humidified atmosphere containing 5% CO_2. (Patel S., *et al.* 2023) [20]. Cells were seeded into 6-well culture plates at a density of approximately 1×10^5 cells per well and incubated overnight to allow cell attachment. Once the cells reached 70–80% confluence, they were treated with different concentrations of the plant extract and incubated for 24 h. (Khan H., *et al.* 2023) [11]. Untreated cells cultured under identical conditions served as the control group.

Following treatment, the cellular morphology was examined using an inverted phase-contrast microscope. Morphological characteristics associated with cytotoxicity and apoptosis were evaluated by comparing treated cells with control cells. (Singh R., *et al*. 2024) [27]. Typical apoptotic features such as cell shrinkage, membrane blebbing, cell rounding, and detachment from the culture surface were carefully recorded. (Zhang X., *et al.* 2024) [31]. Representative images of both control and treated cells were captured at suitable magnifications to document the morphological alterations induced by the plant extract. (Kooti W., *et al*. 2021) [12].

15. Statistical Analysis

All experimental measurements were performed in triplicate to ensure the reliability and reproducibility of the results. The obtained data were expressed as mean ± standard deviation (SD). (Kumar S., *et al.* 2024) [13].Statistical analysis was conducted using GraphPad Prism software. Differences among experimental groups were evaluated using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's multiple comparison test was applied as a post hoc analysis to determine the statistical significance between control and treated groups. This statistical approach was used for the analysis of antioxidant assays, cytotoxicity studies, and other experimental parameters included in the study. (Kumar S., *et al.* 2024) [13]. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

Quantitative Phytochemical Analysis

The quantitative phytochemical analysis demonstrated a differential distribution of bioactive constituents between the leaf and stem extracts of *Heliotropium marifolium*. The ethanol extract derived from the leaves exhibited a comparatively higher concentration of total phenolic compounds and flavonoids than the corresponding stem extract. In addition, both plant parts contained detectable levels of alkaloids and tannins, although their abundance varied between tissues. The elevated accumulation of phenolic and flavonoid compounds in the leaf extract suggests a stronger antioxidant potential relative to the stem extract. These phytoconstituents are widely recognized for their capacity to neutralize reactive oxygen species and modulate oxidative stress–related cellular damage. Consequently, the phytochemical profile of *H. marifolium* leaves indicates their potential relevance as a natural source of antioxidant compounds that may contribute to the prevention or mitigation of oxidative stress–associated disorders, including cancer.

Table 1: Quantitative Determination of the Phytochemicals Present in ethanol extract of Leaf and stem of *Heliotropium marifolium.*

S.No.	Phytochemical	*Heliotropium marifolium* extracts	
		Leaves (mg/g)	Stem (mg/g)
1	Total alkaloids	10.6±0.07	7.32±0.03
2	Flavonoids	8.33±0.16	5.21±0.42
3	Tannins	5.17±0.11	3.22±0.26
4	Saponins	7.24±0.14	5.12±0.40
6	Total phenolics	2.72±0.05	1.71±0.21
7	Total Carbohydrates	0.63±0.14	0.35±0.14
8	Glycosides	3.42±0.22	2.17±0.19
9	Vitamin C	3.29±0.21	2.15±0.15
10	Vitamin E	1.21±0.09	0.92±0.11
11	Proteins	1.26±0.23	0.88±0.17
12	Amino acids	1.92±0.17	0.87±0.19

In vitro Antioxidant Activity

The ethanol extracts obtained from the leaves and stems of *Heliotropium marifolium* demonstrated notable *in vitro* antioxidant capacity in a concentration-dependent manner across several free radical scavenging models, including DPPH, ABTS, hydrogen peroxide, superoxide, hydroxyl, and nitric oxide assays. Among the tested plant parts, the leaf extract consistently produced lower IC_{50} values compared with the stem extract, indicating a stronger radical scavenging potential. This enhanced activity may be associated with the relatively higher levels of phenolic and flavonoid constituents detected in the leaf extract. Furthermore, the antioxidant performance of the extracts approached that of the standard antioxidant, Ascorbic acid, suggesting that *H. marifolium* possesses considerable natural antioxidant capacity. These findings highlight the potential of this plant species as a source of bioactive compounds capable of mitigating oxidative stress, a key factor implicated in the initiation and progression of cancer.

Table 2: Free radical scavenging activity of *Heliotropium marifolium*

Concentration µ g	DDPH	ABTS	H2O2	SUPEROXIDE	OH	Nitric oxide
Stem extract (SE) 50µg	12.31±0.38	12.51±0.50	14.51±0.55	16.62 ±0.51	13.24±0.41	17.60±0.44
SE 300µg	72.37±1.40	80.11±1.52	80.72±1.52	82.73±1.57	78.40±1.37	85.40±1.53
Leaf extract (LE) 50µg	24.39±0.51	18.62±0.81	24.65±0.60	18.52±0.67	16.52±0.55	17.60±0.44
LE 300µg	88.32±1.66	88.40±1.88	91.29±1.70	87.50±1.69	83.72±1.55	85.40±1.53
Ascorbic acid 50µg	30.68±0.89	25.13±0.83	36.52±0.89	28.62±0.80	25.33±0.60	34.41±0.70
Ascorbic acid 300µg	95.47±1.85	92.70±1.82	96.40±1.75	93.45±1.78	90.19±1.70	96.09±1.85

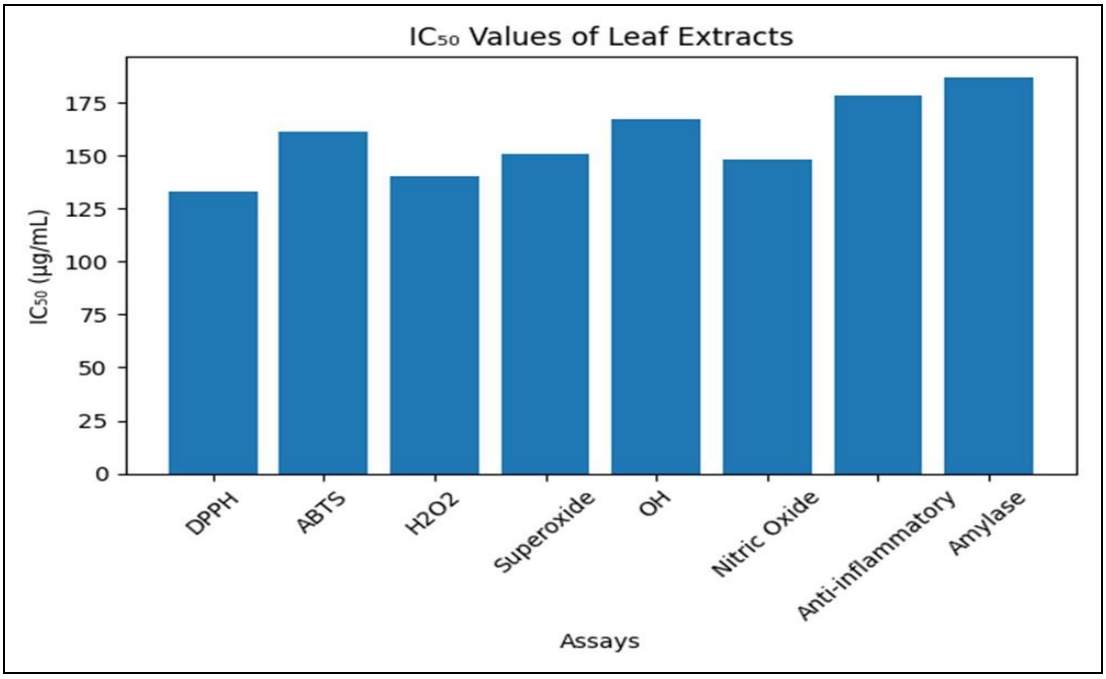

Fig 1: The bar graph presents the IC_{50} Values (µg/mL) of various biological and antioxidant assays for the leaf extract of *Heliotropium marifolium.*

The cytotoxic Activity against MCF-7Cells

The cytotoxic potential of the ethanol extract of *Heliotropium marifolium* was assessed against human breast cancer cells using the MTT cell viability assay. The extract exhibited a concentration-dependent reduction in the viability of MCF-7 cells, indicating a measurable inhibitory effect on cancer cell proliferation. At a concentration of 250 µg/mL, the plant extract produced 72.31% inhibition of cell growth, demonstrating considerable cytotoxic activity. In contrast, the reference chemotherapeutic agent Doxorubicin showed 92.44% inhibition at the same concentration, reflecting a stronger ant proliferative effect. Although the standard drug displayed higher potency, the observed cytotoxic response of *H. marifolium* extract suggests the presence of biologically active phytochemicals capable of suppressing tumour cell growth. These findings indicate that the plant extract may serve as a promising source of natural compounds with potential relevance in breast cancer therapeutic research.

Table 3: Cytotoxic activity of *Heliotropium marifolium* leaf ethanol extract against MCF-7 cells (MTT assay).

Group	Concentration (µg/mL)	Cell Viability (%)	Growth Inhibition (%)	IC_{50} (µg/mL)
Ethanol extract of *Heliotropium marifolium* leaf	50	81.47 ± 1.35	18.53	147.8
	100	66.82 ± 1.58	33.18	
	150	49.73 ± 1.41	50.27	
	200	36.15 ± 1.76	63.85	
	250	24.48 ± 1.64	75.52	
Standard drug (*Doxorubicin*)	50	52.73±1.41	70.24	62.4
	100	39.64 ± 1.63	60.36	
	150	26.73 ± 1.58	73.27	
	200	16.42 ± 1.72	83.58	
	250	9.85 ± 1.51	90.15	

Cytotoxic activity of ethanol extract of *Heliotropium marifolium* against MCF-7 determined by the MTT assay. Data are expressed as mean ± standard deviation (SD) of triplicate experiments. The IC_{50} value represents the concentration required to inhibit 50% of cell viability. Values are expressed as mean ± SD (n = 3).

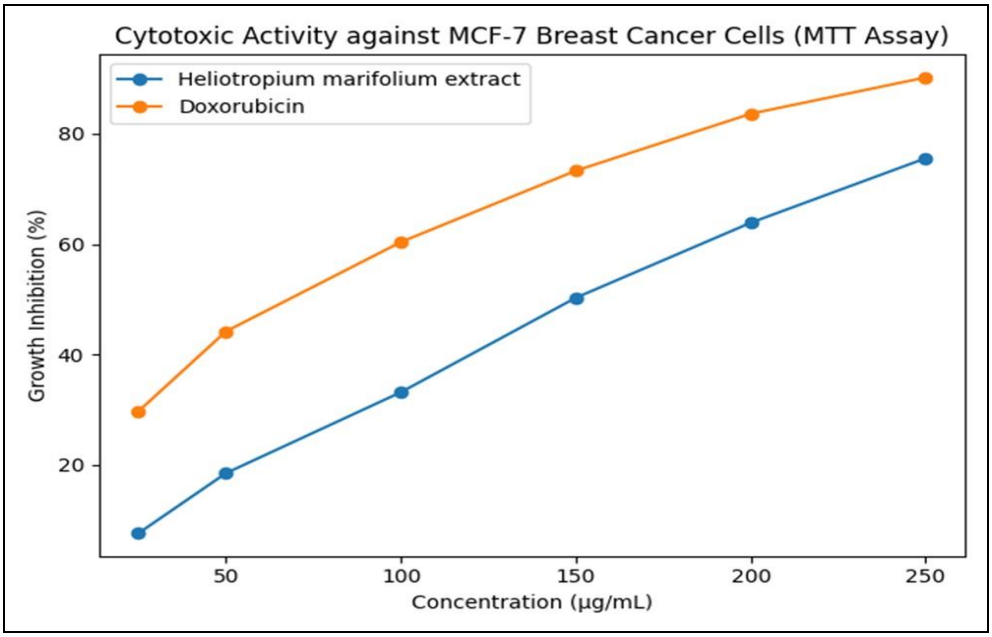

Fig 2: Dose-dependent cytotoxic activity of *Heliotropium marifolium* ethanol extract against MCF-7 cells (MTT assay).

Dose-dependent cytotoxic activity of *Heliotropium marifolium* ethanol extract against MCF-7 determined by the MTT assay, compared with the standard anticancer drug doxorubicin. Increasing concentrations of the extract resulted in progressive inhibition of cell viability.

Comparison of IC_{50} Value

The half-maximal inhibitory concentration (IC_{50}) was calculated to evaluate the cytotoxic potency of the tested samples. IC_{50} represents the concentration of a compound required to reduce 50% of cell viability under the experimental conditions. The ethanol extract of *Heliotropium marifolium* demonstrated an IC_{50} value of 147.8 ± 2.14 µg/mL against the MCF-7 as determined by the MTT assay. For comparison, the reference chemotherapeutic agent Doxorubicin exhibited a significantly lower IC_{50} value of 62.4 ± 1.36 µg/mL, indicating a stronger cytotoxic effect on the cancer cells. Although the standard drug displayed greater potency, the observed inhibitory activity of *H. marifolium* extract suggests the presence of biologically active phytoconstituents capable of suppressing tumour cell proliferation. These findings highlight the potential of the plant extract as a natural source of compounds with possible applications in breast cancer research and anticancer drug discovery

Table 4: IC_{50} Values Comparision of *Heliotropium marifolium* Extract and Standard Drug against Breast Cancer Cells

Sample	Cell Line	IC_{50} value
Heliotropium marifolium leaf extract	MCF-7	147.8±2.14
Doxorubicin	MCF-7	62.4±1.36

The cytotoxic potential of the ethanol extract of *Heliotropium marifolium* leaves against MCF-7 breast cancer cells was assessed using the MTT assay. The findings revealed a clear concentration-dependent reduction in cell viability as the extract concentration increased from 50 to 250 µg/mL. At lower concentrations, the extract exhibited relatively weak inhibitory activity; however, a marked decrease in cell viability was observed at higher concentrations. This trend indicates that the plant extract exerts a significant inhibitory effect on the proliferation of MCF-7 cells. The IC_{50} value of the extract was determined to be 147.8 ± 2.14 µg/mL, while the standard anticancer drug doxorubicin showed a stronger cytotoxic effect with an IC_{50} value of 62.4 ± 1.36 µg/mL. These observations suggest that the bioactive constituents present in *Heliotropium marifolium* may contribute to its anticancer activity, highlighting its potential as a natural source for the development of therapeutic agents against breast cancer.

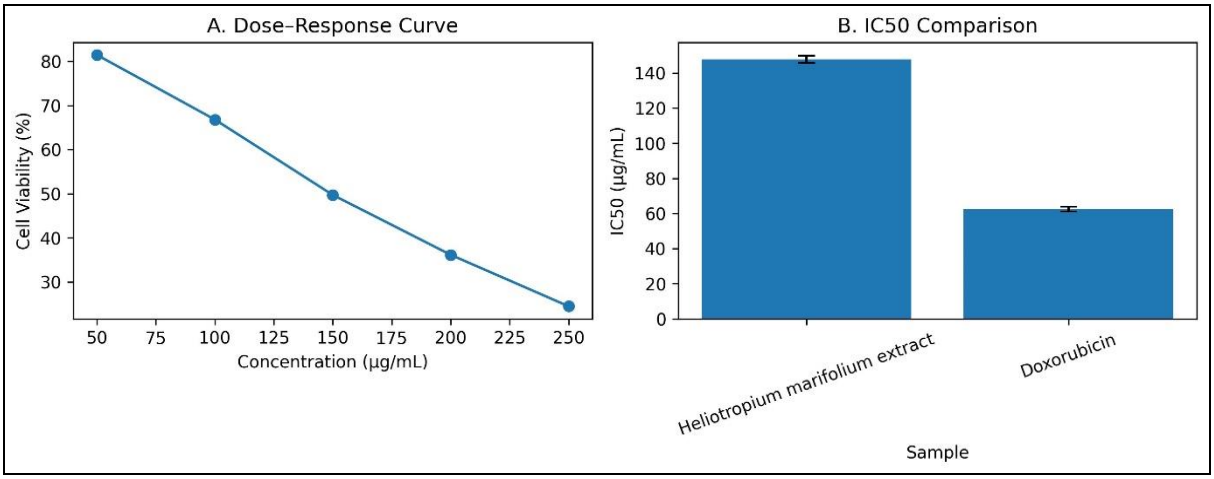

Fig 4: Dose–response curve illustrating the inhibitory effect of *Heliotropium marifolium* leaf ethanol extract on the viability of MCF-7 breast cancer cells determined by the MTT assay.

Morphological Changes in Breast Cancer cells:

Microscopic examination was performed to assess the morphological responses of breast cancer cells following treatment with the ethanol extract of *Heliotropium marifolium*. The untreated control group of MCF-7 displayed typical epithelial characteristics, including a well-spread, polygonal shape with intact plasma membranes and tight intercellular connections. In contrast, cells treated with the plant extract showed noticeable structural alterations indicative of cytotoxic stress. The treated cells progressively lost their characteristic morphology and appeared rounded with reduced attachment to the culture surface. In addition, several hallmark features associated with apoptotic cell death were observed, such as cellular shrinkage, membrane blebbing, and condensation of the cytoplasmic contents. Some cells also exhibited partial detachment and decreased cell density within the culture plate.

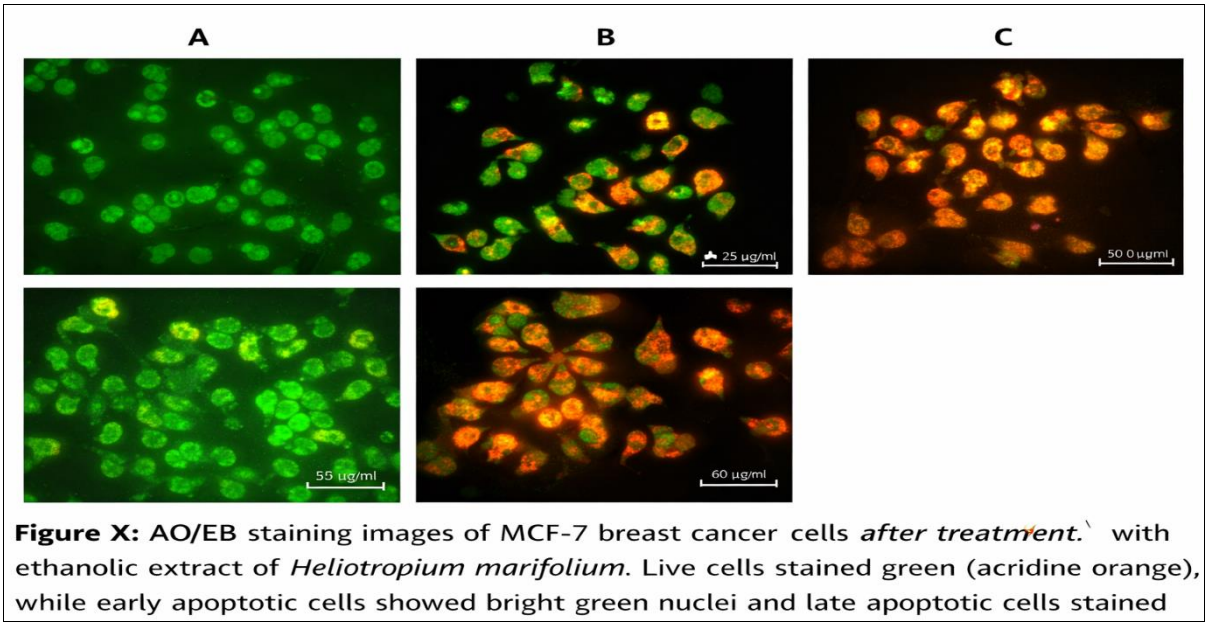

Figure X: AO/EB staining images of MCF-7 breast cancer cells *after treatment.* with ethanolic extract of *Heliotropium marifolium*. Live cells stained green (acridine orange), while early apoptotic cells showed bright green nuclei and late apoptotic cells stained

Fig 5: Morphological Changes in Breast Cancer Cells (MCF-7) After Treatment

These morphological observations demonstrate that exposure to *H. marifolium* extract induces substantial structural disruption in breast cancer cells. The changes are consistent with processes associated with apoptosis or growth inhibition, suggesting that the extract may contain bioactive constituents capable of interfering with the proliferation and survival of malignant cells.

Discussion

Breast cancer remains one of the most frequently diagnosed malignancies among women worldwide and continues to represent a major public health concern. Consequently, considerable research has focused on identifying new therapeutic agents derived from natural sources. Medicinal plants have attracted significant attention because they contain diverse bioactive compounds that may exert antioxidant, anti-inflammatory, and anticancer effects. In the present investigation, the ethanol leaf extract of *Heliotropium marifolium* was evaluated for its phytochemical composition, antioxidant activity, and cytotoxic potential against
MCF-7. Preliminary phytochemical analysis of the extract indicated the presence of several classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenoids. These phytoconstituents have been widely reported to exhibit important pharmacological

properties, particularly in relation to anticancer activity. Among these compounds, flavonoids and phenolic constituents are recognized for their strong antioxidant capacity, primarily through their ability to neutralize reactive oxygen species and reduce oxidative stress–mediated cellular damage. Since oxidative stress plays a crucial role in tumour initiation and progression, the antioxidant properties of these phytochemicals may contribute to the inhibition of cancer cell growth.

The antioxidant activity of *H. marifolium* extract was confirmed through the DPPH radical scavenging assay, which demonstrated a concentration-dependent increase in free radical neutralization. Although the activity of the plant extract was lower than that of the reference antioxidant, Ascorbic acid, the results nevertheless indicate that the extract possesses appreciable antioxidant capacity. This activity is likely associated with the presence of phenolic and flavonoid compounds detected during phytochemical screening.

The antiproliferative effect of the extract was further evaluated using the MTT assay. The findings revealed that the ethanol extract significantly reduced the viability of breast cancer cells in a concentration-dependent manner. Increasing concentrations of the extract resulted in a progressive increase in the percentage of growth inhibition, demonstrating the cytotoxic effect of the plant extract against MCF-7. These results suggest that the bioactive compounds present in the extract may interfere with cellular proliferation pathways in malignant cells.

The calculated IC_{50} value of the extract further supports its inhibitory effect on cancer cell viability. Although the standard chemotherapeutic agent Doxorubicin exhibited a lower IC_{50} value and therefore greater cytotoxic potency, the plant extract still demonstrated measurable anticancer activity. Such findings are consistent with previous reports describing the cytotoxic potential of various medicinal plant extracts against breast cancer cell lines.

In addition to the quantitative cytotoxicity results, microscopic examination of treated cells revealed substantial morphological alterations compared with untreated controls. Cells exposed to the extract exhibited several structural changes commonly associated with apoptotic cell death, including cellular shrinkage, membrane blebbing, rounding of cells, and partial detachment from the culture surface. These morphological features indicate that the extract may induce programmed cell death mechanisms in breast cancer cells.

Collectively, the results obtained in this study demonstrate that *Heliotropium marifolium* possesses notable antioxidant and cytotoxic properties. These biological activities may be attributed to the diverse phytochemicals present in the extract, particularly phenolic and flavonoid compounds. The findings highlight the potential of this plant as a promising natural source of bioactive molecules that could contribute to the development of new therapeutic strategies for breast cancer management. Further studies focusing on the isolation and characterization of active compounds, as well as detailed mechanistic investigations, are necessary to better understand the anticancer potential of this plant.

Conclusion

The present study demonstrates that the ethanol leaf extract of *Heliotropium marifolium* exhibits appreciable antioxidant and cytotoxic activities against MCF-7. Phytochemical analysis confirmed the presence of bioactive constituents, including flavonoids, alkaloids, tannins, saponins, and terpenoids, which may contribute to the observed biological effects. The extract showed notable free radical scavenging activity and inhibited cancer cell proliferation in a concentration-dependent manner, as evidenced by the MTT assay. Morphological changes in treated cells further indicated features consistent with apoptosis. Although the extract displayed lower potency compared with the standard chemotherapeutic agent Doxorubicin, the findings highlight the potential of *H. marifolium* as a promising natural source of anticancer compounds. Further investigations focusing on the isolation of active constituents and mechanistic studies are required to validate its therapeutic potential.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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