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Research Article

APOPTOSIS-INDUCING EFFECTS OF *WRIGHTIA TINCTORIA* LEAF EXTRACT ON HACAT KERATINOCYTES AND A431 SKIN CARCINOMA CELLS

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ABSTRACT

The ethanolic leaf extract of *Wrightia tinctoria* was investigated for its potential anti-psoriatic and anticancer effects using HaCaT keratinocytes and A431 skin carcinoma cells. Cytotoxicity determined by MTT assay demonstrated a concentration-dependent reduction in cell viability, with IC_{50} values of 42.5 µg/mL in HaCaT and 78.44 µg/mL in A431 cells. Morphological changes, including shrinkage, blebbing, and detachment, indicated apoptosis as the underlying mechanism of cell death. Apoptotic induction was further confirmed by acridine orange/ethidium bromide staining, which revealed nuclear condensation and chromatin fragmentation, and by PI staining, which showed a significant proportion of early (14.6%) and late (71.5%) apoptotic cells. DNA fragmentation analysis produced the characteristic laddering pattern, supporting apoptosis as the primary cytotoxic pathway. These observations suggest that the phytoconstituents present in *W. tinctoria* are capable of modulating cellular proliferation and inducing apoptosis in both keratinocytes and carcinoma cells. The findings support the therapeutic relevance of *W. tinctoria* as a natural source of bioactive compounds, with potential applications in the management of psoriasis and skin cancer. Overall, this study highlights the value of *W. tinctoria* as a promising candidate for apoptosis-mediated chemoprevention and therapy.

Keywords: Anticancer activity, Apoptosis, Cytotoxicity, DNA fragmentation, Skin carcinoma, *Wrightia tinctoria.*

INTRODUCTION

Psoriasis is a chronic, recurrent, immune-mediated skin disorder characterized by erythema, scaling, itching, and pain (Xiong *et al*., 2025; Greb *et al*., 2016; Griffiths *et al*., 2021; Parisi *et al*., 2020). This condition significantly impairs patients' quality of life and is frequently accompanied by psychological comorbidities such as anxiety and depression (Kimball *et al*., 2005; Parisi *et al*., 2013). Although its exact etiology remains unclear, psoriasis arises from a complex interplay of genetic susceptibility, immune dysregulation, and environmental factors (Bowcock & Krueger, 2005; Elder *et al*., 2010). As one of the most prevalent chronic inflammatory skin diseases worldwide, psoriasis poses considerable therapeutic challenges due to its systemic involvement and unpredictable clinical course (Campanati *et al*., 2021).

Skin cancer represents the most common malignancy globally, with up to 95% of keratinocyte cancers and most melanomas in fair-skinned populations linked to ultraviolet (UV) radiation, making many cases largely preventable (Garbe *et al*., 2024). Non-melanoma skin cancers (NMSC), primarily squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), show increasing incidence, especially among older adults, with variation influenced by geographic location, gender, and socioeconomic status (Pan *et al*., 2025). SCC is notably prevalent among individuals with albinism due to reduced melanin protection (Iloghalu *et al*., 2025). Originating from epidermal keratinocytes, SCC is associated with chronic UV exposure, advancing age, male sex, immunosuppression, smoking, and genetic predisposition (Catalano *et al*., 2024). While early-stage NMSC is generally curable, about 25% of SCC lesions may progress to more aggressive forms, including multiple

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myeloma (Hasan *et al*., 2023). Surgical excision remains the standard treatment, occasionally requiring reconstructive surgery or lymph node dissection in advanced cases (Shambharkar *et al*., 2021). Given the significant impact of both psoriasis and skin cancer on patient quality of life and the complexities of current treatments, there is an urgent need for novel, effective therapeutic options (Ponikowska *et al*., 2025).

Recently, plant-based topical therapies have gained attention for managing superficial skin cancers due to their immunomodulatory, anti-inflammatory, and antioxidant properties (Kowalski *et al*., 2024). *Wrightia tinctoria*, a plant native to the Indian subcontinent, has a long history in traditional medicine (Thiagarajan *et al*., 2024). Its phytochemical profile—including steroids, triterpenoids, saponins, tannins, flavonoids, glycosides, alkaloids, and polyphenols—confers diverse bioactivities such as anti-inflammatory, antimicrobial, antioxidant, anticancer, antidiabetic, and anti-psoriatic effects (Kale *et al*., 2021). Traditionally, its bark and leaves have been used to treat psoriasis, ulcers, digestive disorders, and diabetes (Srivastava, 2014). Despite this well-documented traditional use and the presence of bioactive compounds, rigorous scientific studies elucidating the efficacy and mechanisms of *W. tinctoria* in treating psoriasis and superficial skin cancers are limited. With growing interest in safer, cost-effective plant-based therapies as alternatives or adjuncts to conventional treatments, systematic evaluation of *W. tinctoria's* therapeutic potential is essential.

This study aims to fill these knowledge gaps by comprehensively assessing the pharmacological effects of *W. tinctoria* extracts in established experimental models. Specifically, *in vitro* cytotoxicity and therapeutic potential were evaluated using HaCaT keratinocytes and A431 skin carcinoma cells to explore the extract's anti-psoriatic and anticancer activities.

MATERIALS AND METHODS

Collection and Preparation of *W. tinctoria* Plant

Healthy leaves were collected from various branches of *W. tinctoria* in the Palakkad district of Kerala and were authenticated by Dr. S.S. Hameed, Scientist 'E' at the Botanical Survey of India, Tamil Nadu (No: BSI/SRC/5/23/2023/Tech – 547). In the laboratory, the leaf samples were washed 2–3 times with running fresh water. The leaf material was then air-dried in the shade. After complete drying, the plant material (500 g) was ground using a mechanical grinder, and the powder was stored in small labeled plastic bags.

Extraction

W. tinctoria was assessed and extracted using ethanol through Soxhlet's apparatus. The solvents were extracted from the extracts using a rotating vacuum evaporator, and the filtrate was stored in a desiccator for future use.

Phytochemical Analysis

The prepared extracts were analyzed for the presence of alkaloids, terpenoids, phenols, saponins, flavonoids, steroids, glycosides, carbohydrates, and proteins using the method described by Selvakumar and Singh (2016), with appropriate modifications made as necessary. The results are displayed as (+ve), indicating presence, and (-ve), indicating absence.

Anti-Psoriatic Activity of Ethanolic Extracts in HaCaT Cells by MTT Assay

The anti-psoriatic activity of ethanolic extracts was evaluated *in vitro* on HaCaT keratinocyte cells using the MTT assay. Cells were cultured in DMEM with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in 5% CO_2. Densities of 1×10^4 to 5×10^4 cells were seeded in 96-well plates and incubated for 5 days. Extracts at 25, 50, and 100 µg/mL were applied for 24 hours. After treatment, MTT reagent was added, and absorbance was read at 450 nm. IC_{50} values were calculated, and cytotoxicity graphs were generated. Dithranol was used as a positive control (10 µg/mL). Morphological changes were observed with Nikon bright-field inverted microscopy.

In Vitro Anticancer Activity

Skin carcinoma (A431) cell lines were procured from the National Centre for Cell Science (NCCS), Pune. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics (penicillin and streptomycin) and maintained at 37°C in a humidified atmosphere containing 5% CO_2 to ensure optimal growth conditions and sterility.

Cell Viability Assay (MTT Assay) in A431 Cells

A431 epidermoid carcinoma cells were seeded in 96-well plates and allowed to adhere overnight. After washing with 200 µL sterile 1X PBS, cells were treated with *W. tinctoria* ethanolic extract at concentrations of 25, 50, 75, 100, and 150 µg/mL in serum-free media. Plates were incubated for 24 hours at 37°C with 5% CO_2. Post-treatment, the medium was removed, and 0.5 mg/mL MTT solution (in 1X PBS) was added. Plates were incubated for 4 hours at 37°C to allow formazan formation by viable cells. The MTT solution was then discarded, cells washed once with PBS, and formazan crystals dissolved in 100 µL DMSO. Absorbance was read at 570 nm. Cell viability was calculated relative to control using the formula:

Cell Viability = (OD control / OD treated) × 100

IC_{50} values were determined by regression analysis.

Morphological Changes in A431 Cells

A431 cells were cultured in sterile 35-mm cell culture Petri dishes and maintained at 37°C in a humidified atmosphere with 5% CO_2. The cells were treated with varying concentrations of *W. tinctoria* ethanolic leaf extract (25, 50, 75, 100, and 150 µg/mL), while doxorubicin (2 µg/mL)

was used as a positive control. Untreated cells served as the nenegative controlsr treatment, morphological alterations in A431 cells were assessed using an inverted phase contrast microscope. The degree of cytotoxicity was evaluated based on observable changes such as cell rounding, shrinkage, detachment, and loss of typical cellular architecture compared to the untreated controls.

Acridine orange/ethidium bromide (AO/EB) staining method

A431 cells were seeded at 5×10^5 cells/well in 12-well plates and incubated overnight at 37°C with 5% CO_2. Cells were treated with *W. tinctoria* ethanolic extract at IC_{50} and incubated for 24 and 48 hours. After treatment, cells were washed twice with PBS. A staining solution containing acridine orange (AO) and ethidium bromide (EB) (100 µg/mL each) was added (1 µL per well) and incubated in the dark at 37°C for 30 minutes. Cells were washed twice with PBS to remove excess dye and immediately observed under a fluorescence microscope within 20 minutes. AO stains all cells green by binding DNA, while EB stains only cells with compromised membranes orange. Fluorescence patterns and nuclear morphology were used to differentiate viable, apoptotic, and necrotic cells.

Cell Cycle and Apoptosis Analysis by Flow Cytometry

The selected A431 cell lines (2 × 106 cells/ml) were treated with IC_{50} concentrations of the material for 24 h each. The cells were harvested using centrifugation, then washed with ice-cold PBS and were later resuspended in ice-cold 70% ethanol overnight. The cells underwent treatment with 50 µg/mL of RNase at 37ºC, were subsequently subjected to centrifugation, and were stained with 100 µg/mL of propidium iodide (PI) for 15 min.-Stained cells were immediately subjected to flow cytometry analyses using a FACS Canto II flow cytometer (BD Biosciences).

DNA fragmentation

Briefly, A431 cells were seeded in six-well plates at 1×10^6 cells/well and incubated for 24 hours at 37 °C in 5% CO_2. After washing with PBS, cells were treated with the IC_{50} concentration in serum-free DMEM and incubated another 24 hours. Cells were trypsinized and centrifuged at 10,000 rpm for 10 minutes, and the pellet was lysed with 500 µL lysis buffer for 1 hour. Next, 700 µL phenol-chloroform-isoamyl alcohol was added, mixed, and centrifuged. The aqueous phase was transferred, mixed with cold isopropanol, and centrifuged again. The DNA pellet was air-dried for 30 minutes and dissolved in 50 µL of distilled water. DNA was quantified by UV spectrophotometry at 260 nm and analyzed on 0.8% agarose gel with a 100 bp ladder. Captured by gel documentation system (BioRad USA).

Statistical Analysis

All *in vitro* experiments were performed in triplicate and repeated at least three times. Statistical analysis was done using SPSS v17.0, with $p < 0.01$ considered significant.

RESULTS AND DISCUSSION

The qualitative phytochemical screening of *W. tinctoria* leaf extract prepared exclusively using ethanol as the solvent revealed the presence of several significant bioactive compounds (Table 1)**.** Alkaloids were detected by Mayer's test, while terpenoids were identified through the copper acetate test. Phenolic compounds were confirmed using the ferric chloride test, and saponins were indicated by the foam test. Flavonoids were also present, as demonstrated by the sodium hydroxide test. The presence of steroids and glycosides was validated by the Salkowski and Keller-Kiliani tests, respectively. Additionally, carbohydrates and proteins were positively identified via Molisch's and Millon's tests. Quinones were absent, as evidenced by the negative Borntrager's test. These results highlight the diverse phytochemical composition of the ethanolic extract of *W. tinctoria* leaves, which may underpin its therapeutic potential. The analysis of leaf extracts from *W. tinctoria* revealed the existence of steroids, flavonoids, fatty acid esters, and terpenes (Khan *et al*., 2021). It was recently reported that qualitative phytochemical analysis identified the presence of both primary and secondary metabolites, such as proteins, phenols, tannins, flavonoids, terpenoids, glycosides, saponins, and coumarins (Sailaja *et al*., 2024).

Table 1. Qualitative analysis of phytochemicals from *W. tinctoria* leaf ethanol extracts.

SL. No.	Phytochemical	Analyzing method	Ethanol extract
1	Alkaloids	Mayer's test	+
2	Terpenoids	Copper acetate test	+
3	Phenols	Ferric chloride test	+
4	Saponins	Foam test	+
5	Flavanoids	Sodium hydroxide test	+
6	Quinines	Borntrager's test	_
7	Steroids	Salkowski test	+
8	Glycosides	Keller-Kiliani's test	+
9	Carbohydrate	Molisch's test	+
10	Protein	Millon's test	+

Present (+), Absent (-)

Anti-Psoriatic Activity of *W. tinctoria* Ethanolic Leaf Extract in HaCaT Cells

The cytotoxic potential of *W. tinctoria* ethanolic leaf extract was evaluated against HaCaT keratinocyte cell lines using the MTT assay. Untreated control cells exhibited negligible cytotoxicity, maintaining viability greater than 99%. Treatment with the extract elicited a dose-dependent increase in cytotoxicity, with 40.3% ± 1.25, 53.9% ± 0.57, and 62.6% ± 0.75 cytotoxicity observed at 25, 50, and 100 µg/mL concentrations, respectively. Corresponding cell viabilities were 59.6% ± 0.75, 46.3% ± 1.25, and 37.3% ± 1.25, respectively **(**Table 2 and Figure 1**).** The calculated half-maximal inhibitory concentration (IC_{50}) was 42.5 µg/mL, underscoring the extract's potent anti-proliferative effect on keratinocytes. At the highest tested concentration (100 µg/mL), cytotoxicity levels were comparable to the positive control dithranol (64.9% ± 0.57 at 10 µg/mL).

Morphological evaluation of HaCaT cells treated with *W. tinctoria* ethanol leaf extract for 24 hours showed concentration-dependent effects **(**Figure 2**).** The control group (A) exhibited normal, healthy cell morphology. Cells treated with 25 µg/mL (B) showed slight morphological changes, while treatment with 50 µg/mL (C) resulted in noticeable cellular damage and some dead cells. At 100 µg/mL (D), cells displayed significant cytotoxicity, characterized by increased cell shrinkage and death. The positive control, dithranol at 10 µg/mL (E), showed extensive cytotoxic effects, confirming the assay's validity. These findings collectively suggest that *W. tinctoria* ethanolic leaf extract exerts significant anti-proliferative activity in HaCaT cells, highlighting its potential as a therapeutic agent for psoriasis by targeting keratinocyte hyperproliferation.

Table 2. Cytotoxicity assay of *W. tinctoria* ethanol leaf extract in HaCaT cell lines.

Keratinocyte cell lines – Cytotoxicity Assay			IC_{50}
Concentration of Sample (µg/mL)	Cytotoxicity (%)	Cell viability (%)	IC_{50}
0	0	> 99	No cytotoxicity
25	40.3 ± 1.25	59.6 ± 0.75	IC_{50} = 42.5µg/mL for HaCaT cells
50	53.9 ± 0.57	46.3 ± 1.25	
100	62.6 ± 0.75	37.3 ± 1.25	
10 (positive control dithranol)	64.9 ± 0.57	35.3 ± 0.75	Cytotoxicity

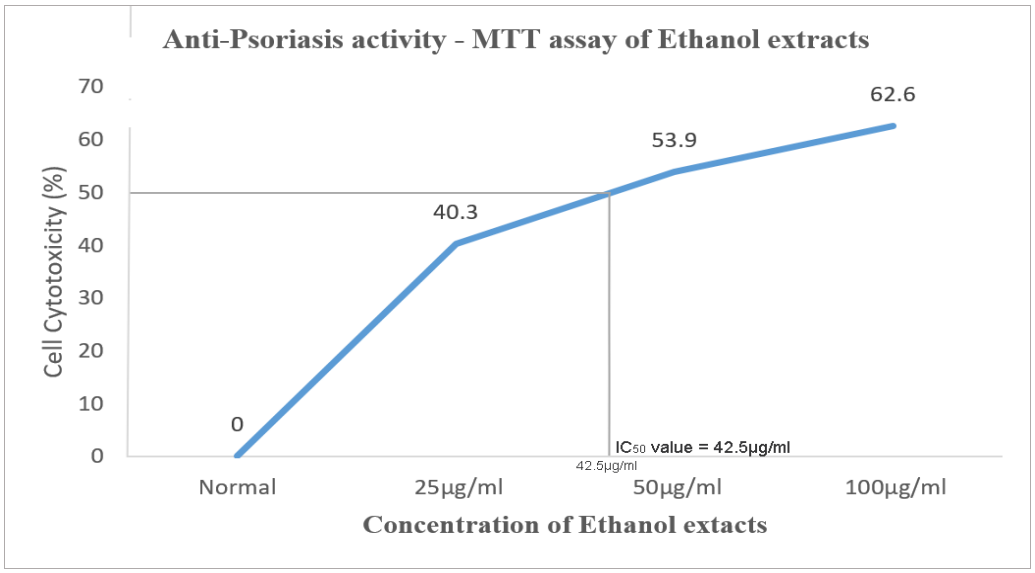

Figure 1. Cytotoxicity assay of *W. tinctoria* ethanol leaf extract in HaCaT cell lines.

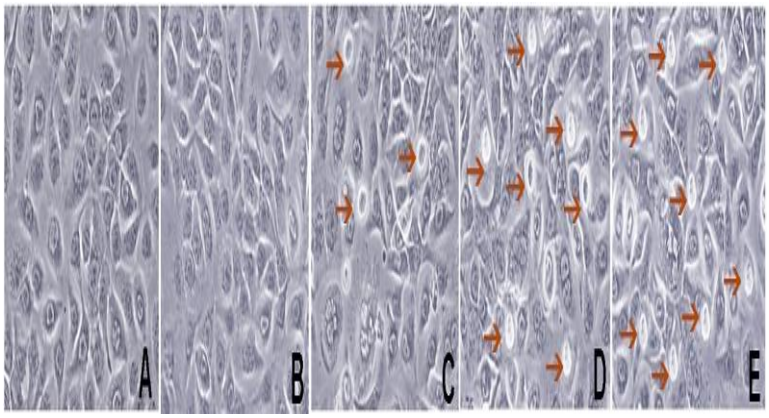

Figure 2. Morphological evaluation of HaCaT cells after 24-hour treatment with *W. tinctoria* ethanol leaf extract at concentrations of (B) 25 µg/mL, (C) 50 µg/mL, and (D) 100 µg/mL. (A) Untreated control cells, and (E) positive control treated with 10 µg/mL Dithranol.

The study demonstrated that *W. tinctoria* possesses notable antiproliferative activity against skin keratinocytes. While the petroleum ether extract of *Crotalaria juncea* (IC_{50} = 45.45 µg/mL) and the ethanol extract of *Leucas aspera* (IC_{50} = 55.36 µg/mL) reported by Singh *et al*. (2015) showed comparatively stronger effects, both *W. tinctoria* oil and its emulgel formulation exhibited significant inhibitory activity with IC_{50} values of 89.64 ± 15.44 µg/mL and 84.72 ± 12.34 µg/mL, respectively. The slightly improved efficacy of the emulgel correlates with recent findings where the formulation achieved a 98.87% *in vitro* clearance of psoriatic lesions within 10 hours (Jurel *et al*., 2024; Magesh *et al*., 2024; Krishna *et al*., 2024). The absence of significant cytotoxicity at 6.25 µg/mL suggests a concentration-dependent response, a characteristic feature of many plant-based therapeutics (Jurel, 2024). These results reinforce the recognition of *W. tinctoria* as a promising medicinal agent, particularly in dermatological conditions such as psoriasis, hair loss, and malignancy.

The MTT assay quantitatively demonstrated that the ethanolic extract of *W. tinctoria* leaves exerts a potent, dose-dependent cytotoxic effect on A431 human skin carcinoma cells. Cell viability showed a progressive decline with increasing extract concentrations ranging from 25 µg/mL to 150 µg/mL (Figure 3)**.** While viability remained comparatively high at sub-cytotoxic levels (25–50 µg/mL), a pronounced reduction was observed at concentrations above 75 µg/mL, culminating in minimal viability at 150 µg/mL. The half-maximal inhibitory concentration (IC_{50}) was determined to be 78.44 µg/mL, underscoring the extract's efficacy in inhibiting cell proliferation. Comparable cytotoxic effects of *W. tinctoria* have been reported in other cancer models. Thiagarajan *et al*. (2024) demonstrated an IC_{50} of 48.89 µg/mL against oral cancer cells, while doxorubicin, used as a positive control, showed greater potency (IC_{50} = 9.62 µg/mL). Similarly, *W. tinctoria* extract inhibited A431 cells with an IC_{50} of 31.2 µg/mL in an independent study, highlighting variability likely due to differences in extraction methods, experimental conditions, and cell line responses. Further, vanilla leaf extract was also reported to suppress A431 cell proliferation (Vijaybabu and Punnagai, 2019), underscoring the potential of phytochemicals as alternative or adjunct anticancer agents.

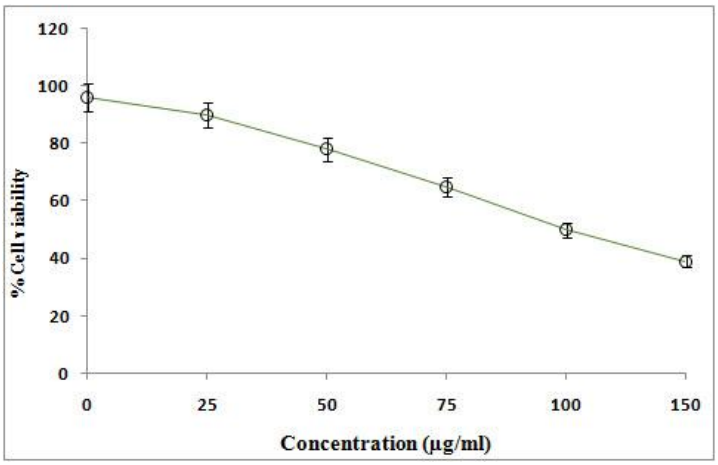

Figure 3. MTT analysis of ethanolic leaf extract of *W. tinctoria*

Morphological assessment via phase-contrast microscopy corroborated these quantitative findings; untreated control cells displayed typical epithelial morphology with intact plasma membranes and confluent monolayers. Treatment with 25 and 50 µg/mL concentrations induced mild cytopathic effects, including reduced cell density and slight membrane blebbing. Elevated concentrations (75–150 µg/mL) elicited pronounced cytotoxicity characterized by cellular shrinkage, membrane disruption, rounding, detachment, and formation of apoptotic bodies, indicative of programmed cell death pathways activation. The positive control, doxorubicin (2 µg/mL), induced extensive cytolysis and apoptosis, validating assay sensitivity **(**Figure 4 A-G**).** These integrated findings provide compelling evidence for the pro-apoptotic and antiproliferative properties of *W. tinctoria* ethanolic leaf extract, advocating its potential as a bioactive agent in skin carcinoma therapeutics. Similar apoptotic features such as cell shrinkage, detachment, and nuclear condensation have been reported in A431 cells treated with other plant extracts (Nakamura, 2013; Akindele *et al*., 2015; Mohansrinivasan *et al*., 2015; Yajarla *et al*., 2014), reinforcing the role of phytochemicals as promising anticancer agents. Collectively, these findings suggest that *W. tinctoria* ethanolic leaf extract may act through apoptosis-mediated pathways to suppress A431 cell proliferation, supporting its potential as a natural therapeutic candidate for skin carcinoma management.

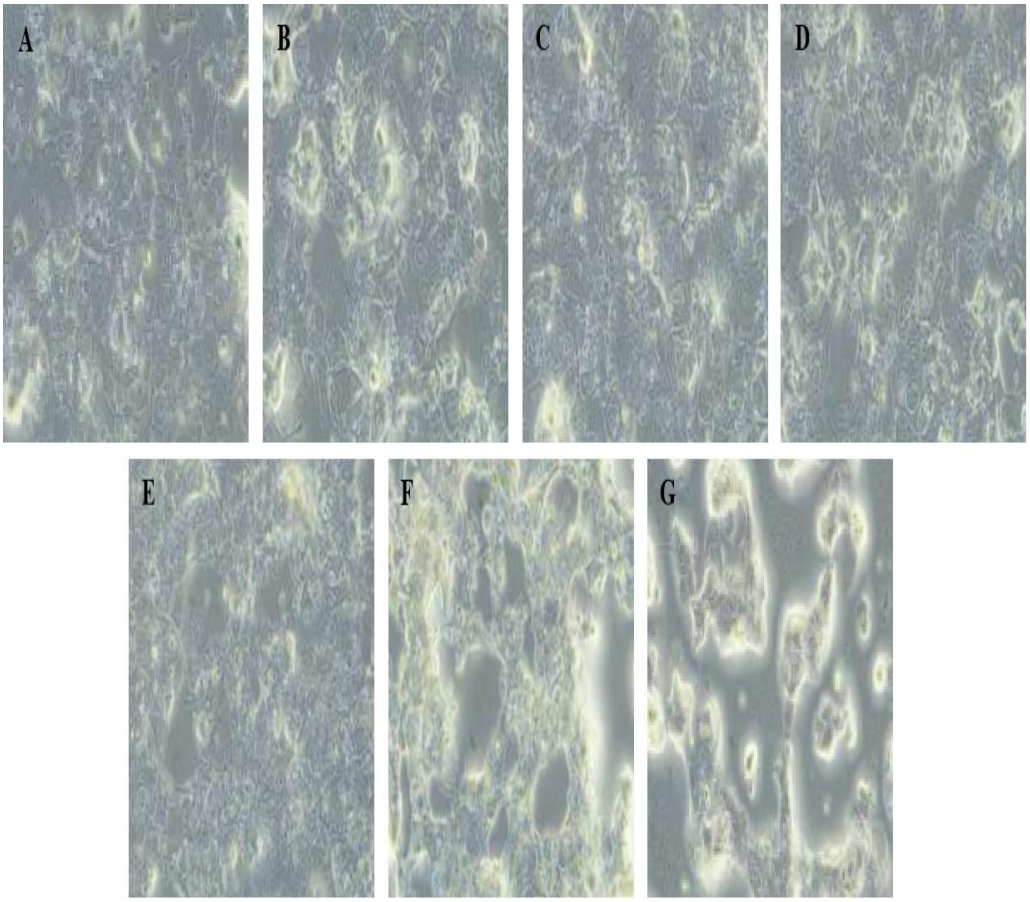

Figure 4. Morphological analysis of ethanolic leaf extract of *W. tinctoria* treated A431 cells for 24 hrs (A) Control (B)25 µg/ml (C) 50 µg/ml (D) 75 µg/ml (E) 100 µg/ml (F) 150 µg/ml (G) 2 µg/ml (Doxorubicin - Positive Control).

The Acridine Orange (AO) and Ethidium Bromide (EtBr) dual staining assay was utilized to evaluate apoptotic induction in A431 cells following treatment with the ethanolic leaf extract of *W. tinctoria* at the determined IC_{50} concentration of 78.44 µg/mL. In untreated control cells (Figure 5 A and B), predominant green fluorescence was observed, indicative of viable cells with intact plasma membranes and minimal membrane permeability to EtBr. Conversely, treated cells **(**Figure 5 C and D**)** exhibited significant orange to red fluorescence, reflecting compromised membrane integrity associated with late apoptotic or necrotic stages. Morphological alterations characteristic of apoptosis, including chromatin condensation, nuclear fragmentation, and cell shrinkage, were clearly evident in treated cells.

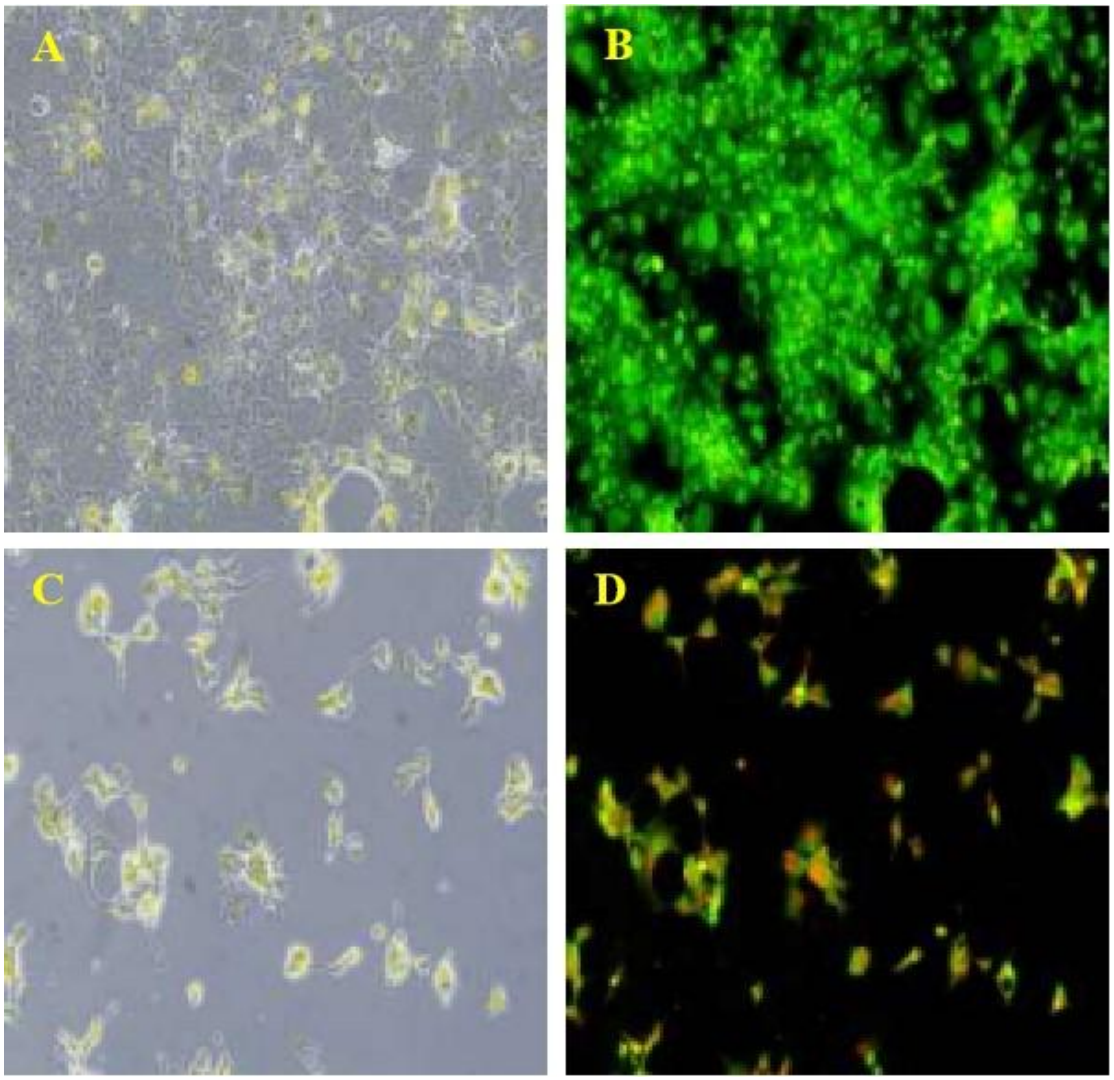

Figure 5. AO/EtBr staining assay of ethanolic leaf extract of *W. tinctoria* treated A431 cells (A and B) Control, (C and D) 78.44µg/ml - IC_{50} Values.

These findings confirm that *W. tinctoria* ethanolic extract induces programmed cell death via apoptotic pathways in A431 carcinoma cells, thereby validating its potential cytotoxic mechanism and therapeutic relevance. These findings align with previous reports where natural compounds triggered apoptosis in carcinoma cells. For instance, Jin *et al*. (2020) demonstrated through AO/EB staining that rosmarinic acid significantly increased apoptotic cell populations in a dose-dependent manner, whereas untreated cells showed no apoptotic features. Similarly, other plant extracts have been shown to induce nuclear condensation, cell shrinkage, and fragmentation in A431 cells (Akindele *et al*., 2015; Mohansrinivasan *et al*., 2015; Yajarla *et al*., 2014).

Flow cytometric analysis using Annexin V/PI staining revealed that untreated A431 cells maintained high viability, with 99% of cells classified as viable and negligible apoptosis or necrosis observed **(**Figure 6 A**).** In contrast, treatment with *W. tinctoria* ethanolic extract at the IC_{50} concentration (78.44 µg/mL) significantly induced apoptosis, as evidenced by a substantial increase in both early apoptotic (14.6%) and late apoptotic (71.5%) cell populations. The percentage of viable cells drastically decreased to 12.5%, while necrosis remained minimal at 1.4% (Figure 6 B). These results clearly demonstrate that the extract effectively triggers programmed cell death, predominantly through apoptosis, thereby confirming its potent cytotoxic effect on A431 carcinoma cells. revious studies have demonstrated its anti-melanoma potential, including inhibition of metastasis and angiogenesis, along with significant cytotoxicity against melanoma cells (Antony *et al*., 2015). The apoptotic mechanism observed in the present study is consistent with earlier reports showing that plant-derived biomolecules can trigger programmed cell death in cancer models. For instance, partially purified peptides from Acanthus ebracteatus were shown to enhance apoptosis pathways through Annexin V assays (Khamwut *et al*., 2019). Such evidence reinforces the role of phytoconstituents as modulators of cell proliferation and apoptosis, supporting the therapeutic relevance of *W. tinctoria* as a natural anticancer agent.

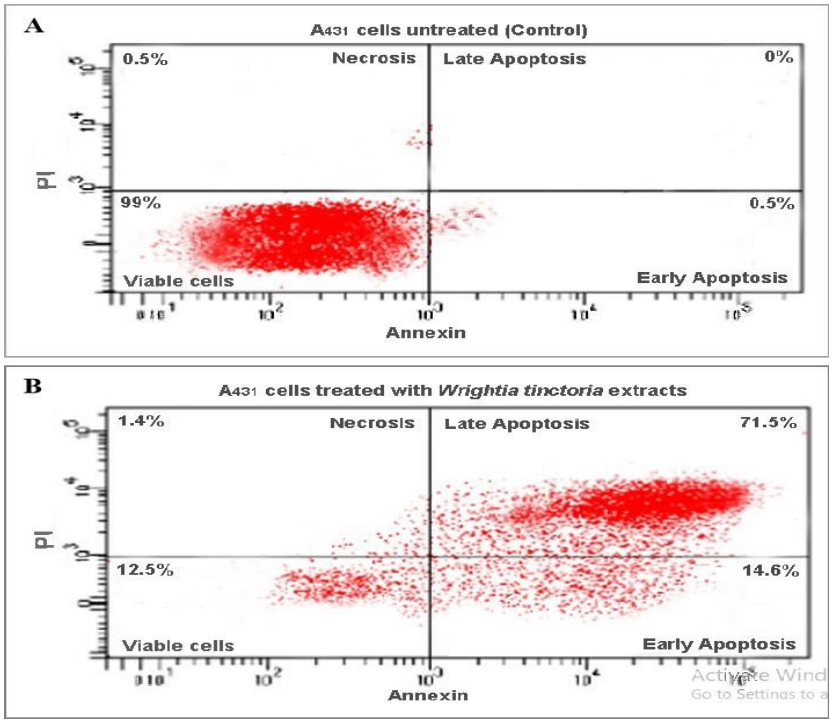

Figure 6. Apoptosis assay by flow cytometry using PI and Annexin V-FITC double staining method ethanolic leaf extract of *W. tinctoria* treated A431 cells (A) Control, (B)78.44µg/ml - IC_{50} Values

The DNA fragmentation assay clearly distinguished between untreated and *W. tinctoria* ethanolic extract-treated A431 cells **(**Figure 7). The untreated control cells (Lane L3) exhibited intact, high-molecular-weight genomic DNA without any signs of fragmentation, indicating normal cellular integrity. In contrast, DNA isolated from the treated cells (Lane L2) showed a distinct laddering pattern, characterized by multiple fragmented bands and smearing, which is indicative of internucleosomal DNA cleavage—a hallmark of apoptosis. The 1 kb DNA ladder (Lane L1) served as a molecular size reference for the fragmented DNA bands. These results confirm that treatment with the ethanolic extract induces programmed cell death through DNA fragmentation, providing strong evidence that the extract promotes apoptosis in A431 carcinoma cells. This mechanism likely contributes to the extract's potent anticancer effects.

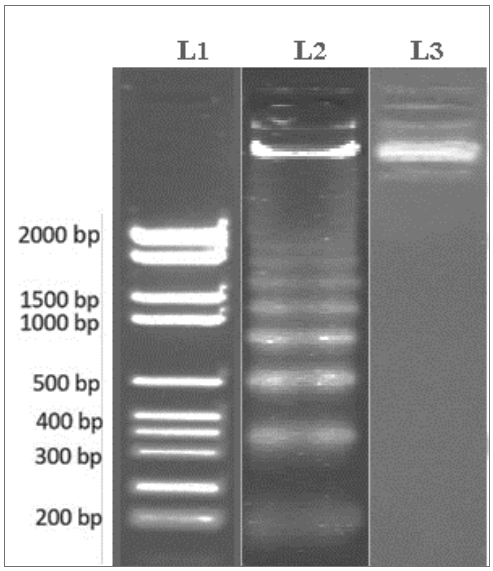

Figure 7. L1: 1 kb DNA marker; L2: Fragmented DNA of A431 cells treated with ethanol extract of the *W. tinctoria*; L3: DNA profile of untreated A431 cells (undigested).

The DNA fragmentation assay provided further evidence of the pro-apoptotic activity of *W. tinctoria*. Treatment with 59 µg/mL of the extract produced a characteristic ladder pattern, while Western blotting confirmed the presence of cleaved caspase-3, indicating activation of apoptosis pathways (Thiagarajan *et al*., 2024). DNA laddering is a well-established marker of apoptosis and offers a rapid means to detect programmed cell death. Similar findings were reported by Singh Charanjeet *et al*. (2019), where expanded DNA fragmentation was observed in A549, U343, and HeLa cells following treatment with dichloromethane and ethyl acetate fractions or methotrexate, whereas vehicle controls showed no fragmentation. These observations not only confirm the apoptotic mechanism of *W. tinctoria* extract but also support the broader notion that natural products remain valuable sources of anticancer agents due to their abundance of bioactive compounds, accessibility, safety, and relatively low cost (Cos *et al*., 2006).

CONCLUSION

The study demonstrated that the ethanolic leaf extract of *W. tinctoria* exhibits a rich phytochemical profile and induces significant cytotoxic and pro-apoptotic effects in both normal and carcinoma cell lines. Morphological alterations, AO/EB staining, flow cytometry, and DNA fragmentation assays confirmed apoptosis as the primary mechanism of action, characterized by nuclear condensation, chromatin fragmentation, and DNA laddering. These findings highlight the therapeutic potential of *W. tinctoria* as a promising source of natural bioactive compounds for managing skin disorders and cancer. Further studies focusing on bioactive compound isolation and mechanistic validation *in vivo* are warranted to advance its therapeutic prospects.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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