Vol 6 No 3 September 2025 Pages 8-18 e-ISSN 2708-5694

Journal of Agriculture, Food and Environment (JAFE)

Journal Homepage: https://journal.safebd.org/index.php/jafe

https://doi.org/10.47440/JAFE.2025.6302

*Research Article*

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# Cancer inhibition, apoptosis induction and wound healing modulation potentials of Halfa Bar (*Cymbopogon proximus*) extracts

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**Article History**

**Received: 07 June 2025**

**Accepted: 30 August 2025**

**Published online: 30 September 2025**

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**Keywords**

Halfa Bar, Cymbopogon proximus, Anticancer, Apoptosis, Wound Healing

**How to cite:** El-Sheikh MM, Hassan RA, Sanad MI and El-Khateeb AY 2025: Cancer Inhibition, Apoptosis Induction and Wound Healing Modulation Potentials of Halfa Bar (*Cymbopogon proximus*) Extracts. J. Agric. Food Environ. 6(3): 8-18.

**A B S T R A C T**

The study on *Cymbopogon proximus* (Halfa Bar) investigated its anticancer, apoptotic, and wound-healing activities through a series of in vitro experiments. Methanolic and ethyl acetate extracts were prepared and tested on MDA-MB-231 breast cancer cells using the MTT assay, which revealed moderate dose-dependent cytotoxicity with $IC_{50}$ values of 155.13 µg/mL (methanolic extract) and 159.24 µg/mL (ethyl acetate extract). Flow cytometry with Annexin V-FITC/7-AAD staining confirmed that the methanolic extract induced apoptosis in a concentration-dependent manner, with higher doses causing significant early and late apoptosis. Transmission electron microscopy further supported apoptotic induction by showing nuclear condensation, membrane blebbing, and apoptotic body formation. In contrast, the wound healing (scratch) assay demonstrated that while control cells achieved 37.1% wound closure after 24 hours, cells treated with $IC_{25}$ and $IC_{50}$ concentrations of the methanolic extract showed inhibited migration and even increased wound size, indicating impaired healing capacity at cytotoxic doses. Overall, the results highlight that *C. proximus* extracts exert significant anticancer and pro-apoptotic effects, but their wound-healing efficacy is dose-dependent and limited by cytotoxicity at higher concentrations.



## INTRODUCTION

Medicinal plants have long served as a cornerstone in the development of therapeutic agents, offering a vast reservoir of bioactive compounds with diverse pharmacological properties. Among these plants, *Cymbopogon proximus* (commonly known as Halfa Bar) has gained increasing attention in recent years due to its promising therapeutic potential. Belonging to the Poaceae family, *C. proximus* is an aromatic grass widely distributed in North and East Africa, particularly in Egypt, Sudan, and parts of the Arabian Peninsula, and has been traditionally used for treating various ailments, including fevers, digestive disorders, and kidney problems (Ahmed *et al.*, 2022).

Phytochemically, *C. proximus* is rich in essential oils and secondary metabolites such as flavonoids, phenolic acids, terpenoids, and alkaloids, many of which are known for their strong antioxidant properties (Gendy *et al.*, 2021). Antioxidants play a crucial role in neutralizing reactive oxygen species (ROS), which are implicated in aging and the pathogenesis of chronic diseases such as cancer, neurodegeneration, and cardiovascular conditions (Lobo *et al.*, 2010). Recent studies have confirmed that extracts from *C. proximus* exhibit significant free radical scavenging activity, making it a promising natural source of antioxidant agents (Abdelghany *et al.*, 2023).

The antimicrobial potential of *C. proximus* has also been widely investigated. Essential oils extracted from its leaves and stems contain compounds such as piperitone, limonene, and carvone, which have demonstrated strong inhibitory effects against a wide range of Gram-positive and Gram-negative bacteria as well as fungal pathogens (El-Kased *et al.*, 2020). This aligns with growing interest in plant-based antimicrobials as alternatives to synthetic antibiotics, particularly in the face of rising antimicrobial resistance.

Notably, *C. proximus* has also shown promising cytotoxic activity against various cancer cell lines. Experimental

studies have indicated its ability to induce apoptosis in cancer cells, potentially through mechanisms involving mitochondrial disruption, caspase activation, and downregulation of anti-apoptotic genes (Ismail *et al.*, 2021). These findings position *C. proximus* as a potential candidate for natural anticancer therapies, either alone or in combination with conventional treatments.

Furthermore, wound healing is another biological activity attributed to *C. proximus*. The plant's phytoconstituents, particularly phenolics and flavonoids, are believed to promote tissue regeneration by modulating inflammatory responses, enhancing collagen synthesis, and improving re-epithelialization (Mohamed *et al.*, 2022). The integration of plant-based therapies in wound management is gaining traction due to their biocompatibility, cost-effectiveness, and reduced risk of side effects.

Despite these promising findings, comprehensive scientific investigations that link the phytochemical composition of *C. proximus* to its diverse biological activities are still limited. This study aims to fill that gap by conducting a detailed phytochemical profiling of *Halfa Bar* and evaluating its anticancer, pro-apoptotic, and wound healing potentials through in vitro and possibly in vivo models. The outcomes of this research may contribute to the development of novel phytotherapeutic agents derived from *Cymbopogon proximus* for use in modern medicine.

## MATERIALS AND METHODS

The primarily used part of Halfa Bar (*Cymbopogon Proximus*) plant is the naturally dried leaves, due to their bioactive polyphenolic contents, which is the main chemical component. The experimental section for Halfa Bar which purchased from local market at Mansoura City, Mansoura, Egypt, includes various preparations and analytical procedures which were performed during the course of this investigation. This section included the following subtitles:

### Sampling and extraction of plant materials

Sample leaves were conveyed directly to the Agricultural Biochemistry Laboratory, Faculty of Agriculture, Mansoura University, Mansoura, Egypt, and ground into a fine powder and the following transactions according to (Kalaskar *et al.*, 2025). The crushed leaves were extracted by soaking for five times with methanol (20L) at room temperature. The methanolic extract was concentrated almost to dryness under reduced pressure using a rotary evaporator at 45°C to obtain the crude methanolic extract. A weight of (120g) of crude methanolic extract was dissolved for successive extraction in methanol, then distilled water was added in a ratio of (1:2). A separating funnel was used to separate each fraction using polarity gradient solvents such as hexane, methylene chloride, ethyl acetate, and butanol.

### Cell culture

Cells and all materials used in the study were supplied by the American Type Culture Collection (ATCC). Initially, the cells we examined were taken from the pleural effusion of a patient with invasive ductal carcinoma. These MDA-MB-231 cells typically measure 18.9±0.4 µm across and show very strong and spreadable characteristics. All cells were cultured in DMEM media, with 2 mM glutamine, 15% FBS, and sterilized penicillin (100 IU/mL) and streptomycin (100 µg/mL) (Tyagi & Mani, 2023 and Oestmann & Lewis, 1996). All embryos were raised at a temperature of 37°C with a humid atmosphere and 5% $CO_2$. Each time the cells occupied the flask, every three days the culture was moved to another container, now containing up to 1-3×$10^4$ cells/$cm^2$. All the reagents were purchased for cell culture from Gibco (Carlsbad, California).

### Cell Viability Assay (MTT)

The MTT assay is an abbreviation for the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, is a colorimetric method used to assess cell viability, proliferation, and cytotoxicity. It works by measuring the metabolic activity of cells through the reduction of a yellow tetrazolium salt (MTT) to a purple formazan product by mitochondrial enzymes in living cells. Halfa Bar (*Cymbopogon Proximus*) extracts were examined for their impact on the viability of MDA-MB-231 breast cancer cells by using the MTT assay (Kumar *et al.*, 2018). The number of cells in each well in a 96-well plate was 5×$10^4$, and 100 µL of culture medium was used. The plate was incubated overnight to allow cell attachment. Following the growth of the cells, different Halfa Bar extract doses (62 to 2000 µg/mL) were added and incubated overnight on them. When the culture medium was taken out, each well received 10 µL of MTT (5 mg/mL) before the plate was incubated at 37°C in the dark for 4 hours. The formazan crystals were washed in DMSO after they had hatched. An absorbance reading was made at 570 nm on a Thermo Fisher Scientific microplate spectrophotometer. The viability of cells was judged using the untreated control group as the reference, with both set to 100%. We performed every concentration in duplicate, and the averaged absorbance readings showed the percentage of cells alive. Data analysis produced a dose-response curve that made it possible to calculate the $IC_{50}$ from the 24-hour results. For simplicity, we can summarize the representation of the cell culture and MTT assay procedure according to (Mani *et al.*, (2023) as follow in Figure (1).

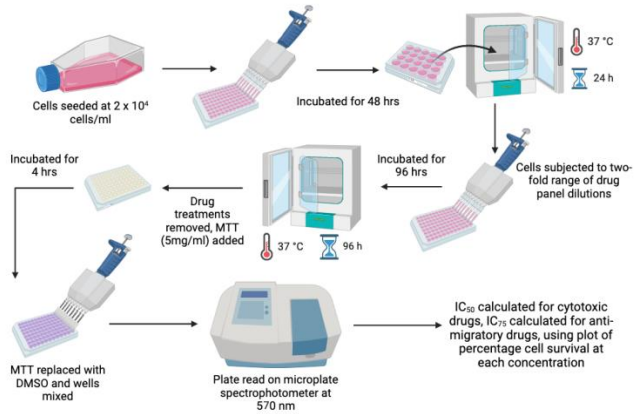



**Figure 1:** Schematic representation of the cell culture and MTT assay procedure.

### Apoptosis Assay

7-Aminoactinomycin D and Annexin V-FITC staining allow for detailed examination of the apoptotic influence of Halfa

Bar extracts on breast cancer cells. The cells were removed after 24 hours of treatment with various Halfa Bar extracts, stained with the recommended BD Pharmingen FITC Annexin V Apoptosis Detection Kit I and then prepared following the method suggested by the producers (Boldt *et al*., 2006). Flow cytometry was accomplished using a BD FACSCanto II flow cytometer (BD Biosciences) and the results were examined on FACSDiva software (Rimac *et al*., 2021). It was possible to tell which cells were in early apoptosis, late apoptosis, necrosis, alive or dead by looking at the dot plot results. The data provided by the assay made it possible to correctly determine when apoptosis began following different exposures to the Halfa Bar extracts.

### Wound Healing (Scratch) Assay

Wound healing activity of Halfa Bar extracts was assessed by a routine *in vitro* scratch assay (Fronza *et al*., 2009 and Varankar & Bapat, 2018). MDA-MB-231 human breast carcinoma cells were plated in 6-well culture plates and incubated to acquire ~90% confluency and develop a homogenous monolayer. A linear scratch (wound) was formed across each well in the cell monolayer using a sterile 200 µL tip of a micropipette. Wells were gently washed with Phosphate Buffer Saline (PBS) to cleanse detached cells and cell debris. The cells were further treated with Halfa Bar extracts at the following concentrations: $IC_{25}$ (~60.5 µg/mL) and $IC_{50}$ (~121.1 µg/mL), which had been established through MTT assay. An untreated group of wells was taken as a control. The wound area images were taken at 0 hours ($T_0$) and following 24 hours ($T_{24}$) incubation in conventional culture conditions (37°C, 5% $CO_2$). The images were captured with an inverted microscope with a calibrated imaging system, and the area of wounds was quantified with ImageJ software. Wound closure percentage was determined with the following formula: Wound Closure (%) = (Area $T_0$ - Area $T_{24}$)/(Area $T_0$) × 100. Each condition was examined with three replicates. The mean wound areas at $T_0$ and $T_{24}$ were measured, and the percentage of wound closure was utilized as a marker of cellular migration and proliferation.

## RESULTS AND DISCUSSION

### Extraction yield quantification

The extraction yield of the crude methanolic extract obtained from the extraction of 2.5 Kg of Halfa Bar leaves by maceration reached 220 grams, while the yield of the successive extraction using polar gradient solvents by using 120g of crude methanolic extract reached 35.0, 14.2, 6.8, and 10.9 grams for each of hexane, methylene chloride, ethyl acetate, and butanol, respectively. The following is a comparative study of the content of these extracts of active compounds, to arrive at the best biological effects for the plant under study.

### MTT Assay of Halfa Bar (Cymbopogon proximus) Methanolic and Ethyl Acetate Extracts

The MTT assay is a widely used colorimetric technique for assessing cell metabolic activity, commonly applied to evaluate cell viability, proliferation, and cytotoxicity. It is based on the ability of metabolically active cells to reduce the yellow tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into insoluble purple formazan crystals via the action of mitochondrial succinate dehydrogenase enzymes. Only viable cells with intact and functional mitochondria can perform this reduction, making the assay a reliable indicator of cell viability. After incubation with MTT, the formazan product is solubilized (commonly using DMSO), and its absorbance is quantified spectrophotometrically, typically at 570 nm. The intensity of the color correlates directly with the number of viable cells (Mosmann, 1983 and Riss *et al*., 2016). Despite its advantages, the MTT assay is sensitive to experimental conditions such as cell density, incubation time, and the metabolic state of cells, necessitating careful standardization for reproducible results. The reaction mechanism can be illustrated as shown in Figure (2) according to Ghasemi *et al*., (2021).

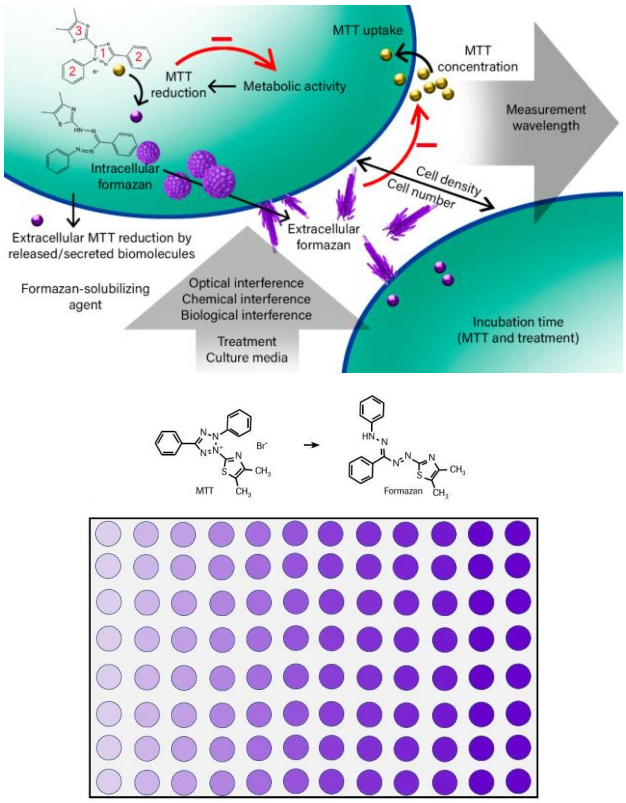



**Figure 2:** The reaction mechanism of the MTT assay.

The cytotoxic potential of Halfa Bar methanolic and ethyl acetate extracts was evaluated using the MTT assay across a range of concentrations (125 to 4000 µg/mL) as shown in Table (1). The optical density (O.D.) values and corresponding cell viability percentages were recorded and used to calculate the half-maximal inhibitory concentration ($IC_{50}$) for each extract. Both extracts exhibited a dose-dependent cytotoxic effect on the tested cells. At the highest concentration tested (4000 µg/mL), the ethyl acetate extract resulted in 20.80% cell viability, while the methanolic extract showed 19.97% viability, indicating strong cytotoxic activity. Correspondingly, the lowest viability was observed at this concentration for both extracts, highlighting their potential to inhibit cell proliferation at high doses.

**Table 1:** Results of MTT assay of Halfa Bar methanolic and ethyl acetate extracts.

| Dose µg/mL | Optical Density [O.D.] | | Cell viability [%] | | $IC_{50}$ [µg/mL] | |
|---|---|---|---|---|---|---|
| | Ethyl acetate extract | Methanolic extract | Ethyl acetate extract | Methanolic extract | Ethyl acetate extract | Methanolic extract |
| ***4000 ppm*** | 0.2140 | 0.2055 | 20.80 | 19.97 | | |
| ***2000 ppm*** | 0.2065 | 0.1875 | 20.07 | 18.22 | | |
| ***1000 ppm*** | 0.2130 | 0.1915 | 20.70 | 18.61 | **159.24** | **155.13** |
| ***500 ppm*** | 0.3590 | 0.1905 | 34.89 | 18.51 | | |
| ***250 ppm*** | 0.8055 | 0.7630 | 78.28 | 74.15 | | |
| ***125 ppm*** | 1.099 | 1.0360 | 106.80 | 100.68 | | |
| **Control** | **0.984** | | **100 %** | | | |

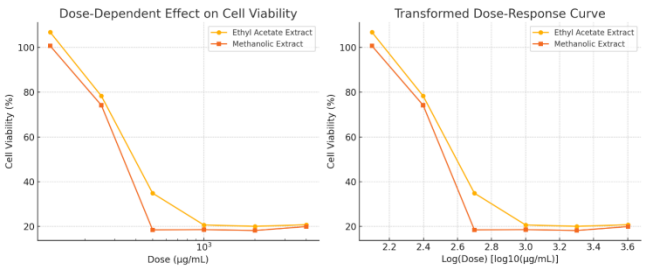
As the concentration decreased, a gradual reduction in cytotoxicity was observed. For instance, at 250 µg/mL, cell viability increased to 78.28% for the ethyl acetate extract and 74.15% for the methanolic extract, and at 125 µg/mL, the cell viability exceeded 100%, indicating potential proliferative or non-toxic behavior at lower doses. The calculated $IC_{50}$ values were 159.24 µg/mL for the ethyl acetate extract and 155.13 µg/mL for the methanolic extract. These values suggest that both extracts possess moderate cytotoxicity, with the methanolic extract being slightly more potent. According to the American National Cancer Institute's (NCI) criteria, crude extracts with $IC_{50}$ values below 20 µg/mL are considered highly cytotoxic, while values between 100–250 µg/mL indicate moderate cytotoxic activity. These findings align with previous studies reporting the anticancer potential of Cymbopogon proximus, likely attributable to its rich content of bioactive secondary metabolites such as flavonoids, phenols, and terpenoids. The slightly higher activity of the methanolic extract may be related to its enhanced ability to extract polar phenolic compounds, which are known to exert pro-apoptotic and cytostatic effects. In conclusion, the MTT assay demonstrates that both methanolic and ethyl acetate extracts of Halfa Bar exhibit moderate but promising cytotoxic effects, warranting further investigation into their mechanisms of action and potential for development as anticancer agents.

The current study evaluated the cytotoxic effects of methanolic and ethyl acetate extracts of *Cymbopogon proximus* (Halfa Bar) using the MTT assay and reported moderate cytotoxic activity with $IC_{50}$ values of 155.13 µg/mL (methanolic extract) and 159.24 µg/mL (ethyl acetate extract). These findings are in line with, and in some aspects expand upon, previous reports on the biological activity of *C. proximus* such as Ahmed *et al.*, (2022), investigated the chemical composition and biological activities of *C. proximus* essential oils and reported notable cytotoxic effects on cancer cell lines. While their study focused primarily on essential oils rather than solvent extracts, they attributed the activity to monoterpenes and sesquiterpenes, such as piperitone and elemol. The current study supports these findings by demonstrating that non-volatile constituents extracted via methanol and ethyl acetate also contribute to cytotoxicity, suggesting a broader range of active compounds beyond volatile oils.

Correspondingly, Gendy *et al.*, (2021), analyzed *C. proximus* extracts and found antiproliferative activity, with $IC_{50}$ values ranging from 140–200 µg/mL, depending on the solvent and the cancer cell type. These values closely match the $IC_{50}$ values obtained in the current study (155.13 and 159.24 µg/mL), reinforcing the moderate cytotoxic potential of the plant's extracts. Also, they emphasized the role of phenolic compounds in the observed effects, consistent with the methanolic extract's slightly stronger activity in the present study. Although, Ibrahim & El-Khateeb (2013) focused on *C. proximus* in the context of renal stone inhibition, this study also touched upon the extract's general biological effects. They reported antioxidant and anti-inflammatory properties, which are often associated with anticancer mechanisms, such as inducing apoptosis and inhibiting oxidative stress-driven proliferation. The current study's findings of dose-dependent cytotoxicity align well with this broader therapeutic profile.

Correspondingly, Mani (2023) and Kumar *et al.*, (2018), studied in comparative herbal involving cytotoxic activity of medicinal plants like *Withania somnifera*, these references reported $IC_{50}$ values of plant extracts between 50–200 µg/mL on various cancer cell lines. The results of the current study fit within this range, placing *C. proximus* among moderately active medicinal plants in terms of antiproliferative potential. This adds value to its consideration as a complementary or supportive anticancer agent.

The present results (Figure 3) are consistent with prior studies that highlighted *Cymbopogon proximus* as a plant with moderate but promising cytotoxic properties, mediated by both volatile and non-volatile phytochemicals. While earlier research focused primarily on essential oils, the current findings expand the evidence base by confirming that solvent extracts (methanol and ethyl acetate) also possess biologically significant anticancer activity. These findings underscore the need for further bio-guided fractionation and mechanistic studies to isolate and characterize the most active constituents responsible for cytotoxicity.




**(a)** **(b)**

**Figure 3:** (a) Dose-dependent effect of halfa bar extracts on cell viability, and (b) Transformed dose-response curve of halfa bar extracts on cell viability.

Dose-dependent effect showed the decrease in cell viability as the dose of Halfa Bar extracts increases, indicating a cytotoxic effect. While, transformed dose-response curve used a log10 transformation of the dose to visualize the response pattern more clearly and assess $IC_{50}$ estimation more accurately.

## Apoptosis activity of Halfa Bar methanolic extract *via* flow cytometry

Apoptosis assay *via* flow cytometry is a powerful technique used to quantify and distinguish between viable, early

apoptotic, late apoptotic, and necrotic cells based on changes in membrane integrity and phosphatidylserine externalization. The most commonly used method involves dual staining with Annexin V conjugated to a fluorochrome (e.g., FITC) and propidium iodide (PI). Annexin V binds with high affinity to phosphatidylserine, which translocates from the inner to the outer leaflet of the plasma membrane during early apoptosis, while PI intercalates into DNA of cells with compromised membranes, marking late apoptotic or necrotic cells. Flow cytometry enables rapid, multiparametric analysis of these fluorescence signals, allowing clear distinction between the four cell populations: viable (Annexin $V^-/PI^-$), early apoptotic (Annexin $V^+/PI^-$), late apoptotic (Annexin $V^+/PI^+$), and necrotic (Annexin $V^-/PI^+$). This method provides high sensitivity and quantitative accuracy in apoptosis detection, making it a standard tool in cancer research and drug screening, the reaction mechanism can be demonstrated as shown in Figure (4) according to Kupcho *et al*., (2019).

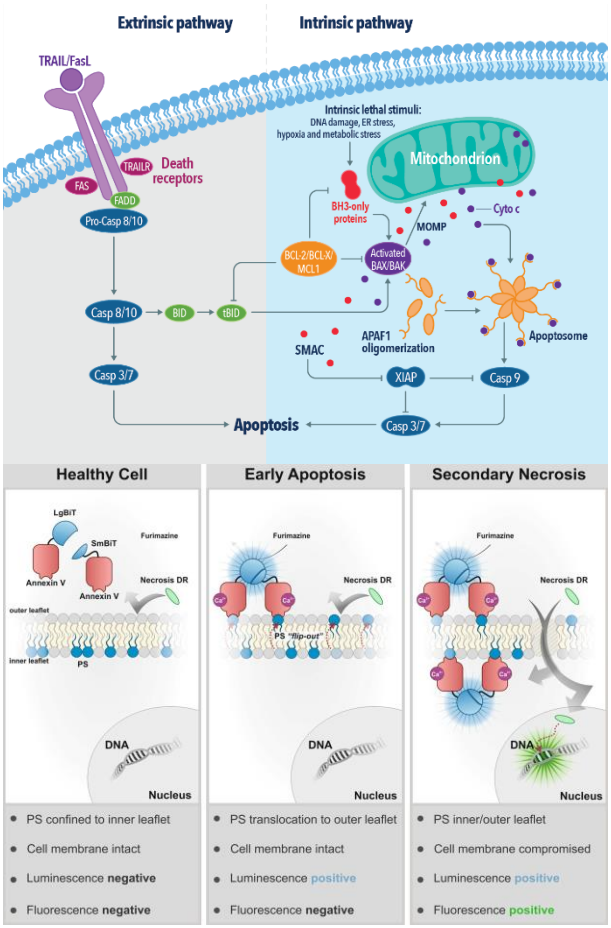
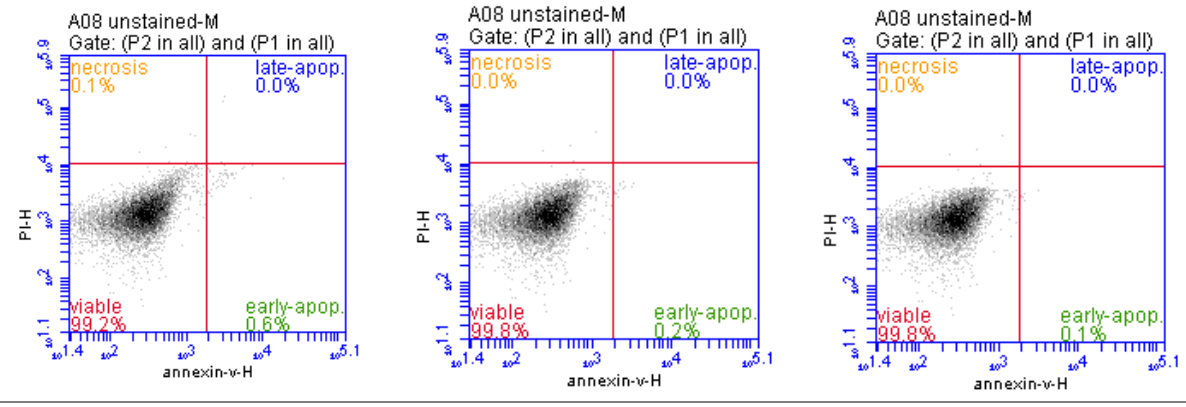



**Figure 4:** The reaction mechanism of apoptosis assay *via* flow cytometry.

Flow cytometry analysis (Panels A-X) was done after Annexin V-FITC and 7-AAD double staining to thoroughly investigate how Halfa Bar crude methanolic extract affects MDA-MB-231 breast cancer cells (Figure 5). Since we could detect staining in different stages of cell death, it was clear to tell the difference between viable, early apoptotic, late apoptotic, and necrotic cells. After 24 hours, control, $IC_{25}$, and $IC_{50}$ samples showed that at higher concentrations, more apoptosis occurred. Most cells in the control group (Panels A-B) were living (Annexin $V^-/7\text{-}AAD^-$) and close to the lower left corner, which suggests that the majority of the cells started viable and with very little apoptosis or necrosis. Once cells were treated with $IC_{25}$ (Panels C-H), there was an increase in early apoptotic cells (lower right quadrant, Annexin $V^+/7\text{-}AAD^-$), so it is reasonable to think that the cells entered the apoptotic pathway from mild cytotoxic stress. Though most cells were still alive, the early signs of apoptosis showed that even small doses of the Halfa Bar extracts could interfere with how cells live. Treatment at $IC_{50}$ levels caused both early and late apoptosis in most cells (Annexin $V^+/7\text{-}AAD^-$ and Annexin $V^+/7\text{-}AAD^+$), and some cells were progressing toward necrosis (Annexin $V^-/7\text{-}AAD^+$). At the highest concentrations, this pattern reveals that apoptosis is more effective, which is consistent with increased damage to the mitochondria and the cell membrane. This pattern and the same outcome being observed in other replicates and over time (Panels O-X) make it more likely that the treatment was responsible for the apoptosis. The results from flow cytometry matched the conclusions from the MTT cytotoxicity assay and the wound healing assay, which found that higher $IC_{50}$ concentrations were better at reducing cell activity and mobility. This may occur when ROS builds, trouble in the mitochondria, and activation of caspase enzymes and proteins from the Bcl-2 family promote more apoptosis. All of these results together prove that Halfa Bar crude methanolic extract has effective pro-apoptotic and anticancer effects, but additional research is needed on how to control its effects on healthy cells.

Halfa Bar crude methanolic extract produces strong cytotoxicity because of several different mechanisms. A main way is the overproduction of reactive oxygen species, which causes oxidative stress, stops the mitochondria from working properly, and leads to apoptosis (Ivanova *et al*., 2016). On the one hand, the information is encouraging, but since the MTT assay only measures mitochondrial activity, it cannot demonstrate every kind of cell death. Hence, it is necessary to perform apoptosis, ROS, and mitochondrial membrane potential tests to identify the methods of cell death.

The presence of Halfa Bar crude methanolic extract leads to apoptosis through the production of oxidative stress, negative effects on mitochondria, and the exciting of apoptosis-related pathways (Sinha *et al*., 2013). The excess ROS causes DNA, lipids, and proteins in the body to be damaged, which may lead to apoptosis (Juan *et al*., 2021). Injury by oxidative stress results in mitochondria enabling cytochrome c to enter the cytosol, which leads to activation of caspase-9 and caspase-3 through the intrinsic apoptosis pathway (Yuan *et al*., 2003). Besides encouraging cancer cells to die, the Halfa Bar crude methanolic extract stops the activity of multiple pathways that support their survival, movement, and ability to invade (Wang *et al*., 2021). All these factors together help bring about dose-dependent apoptosis and slower migration of MDA-MB-231 cells, which further suggests Halfa Bar crude methanolic extract as a useful for the selective treatment of breast cancer.

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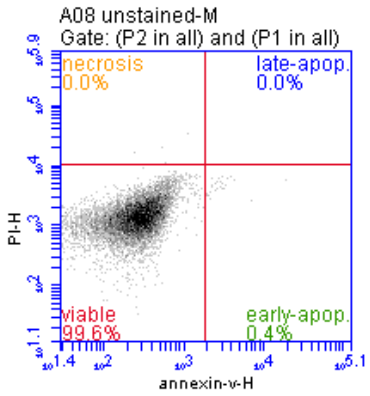
**(C)**

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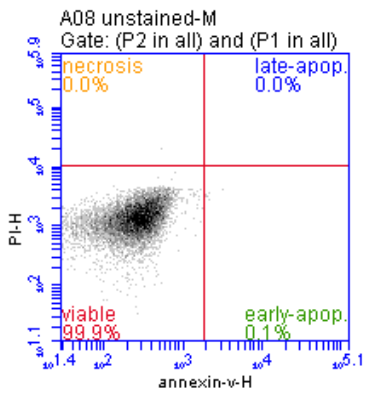


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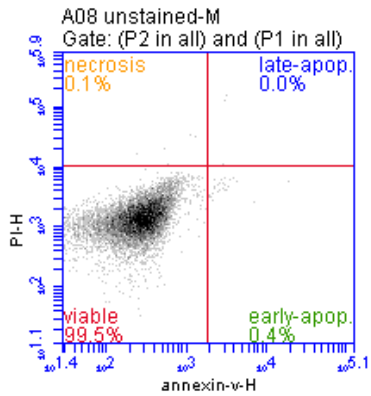


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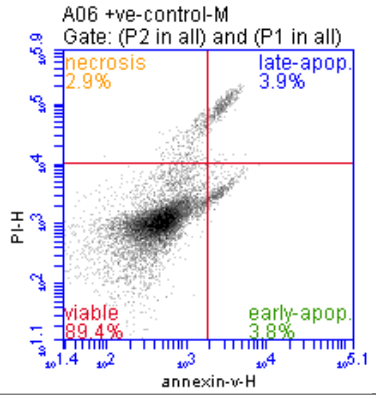


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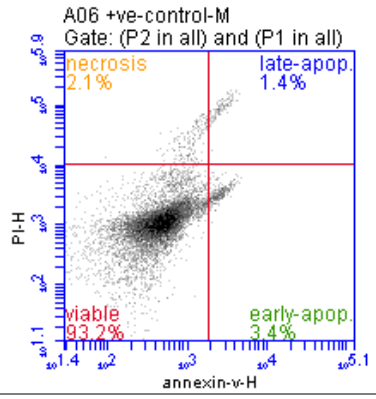


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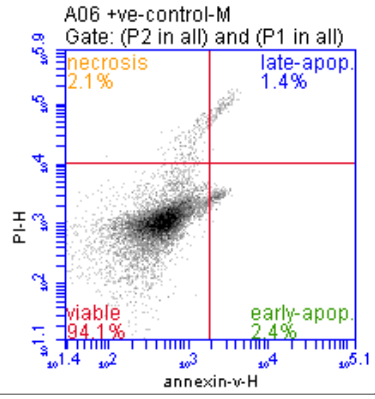


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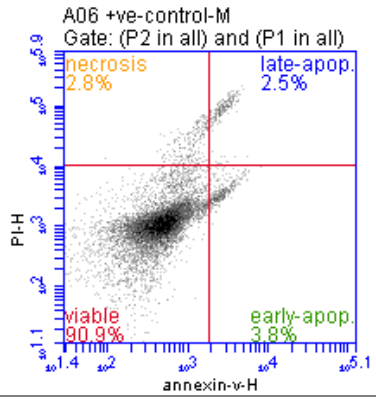


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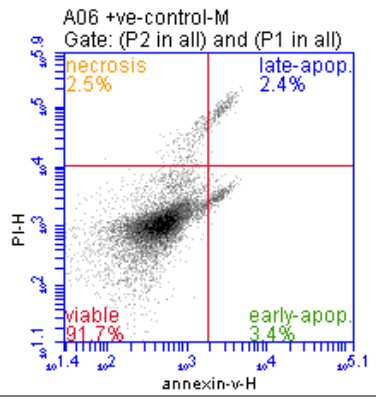


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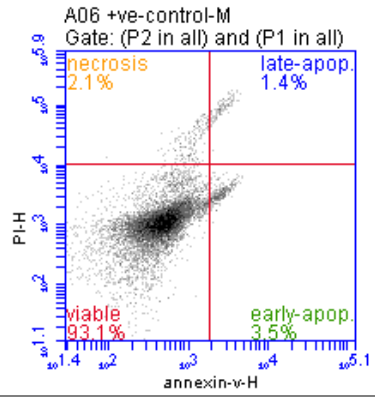


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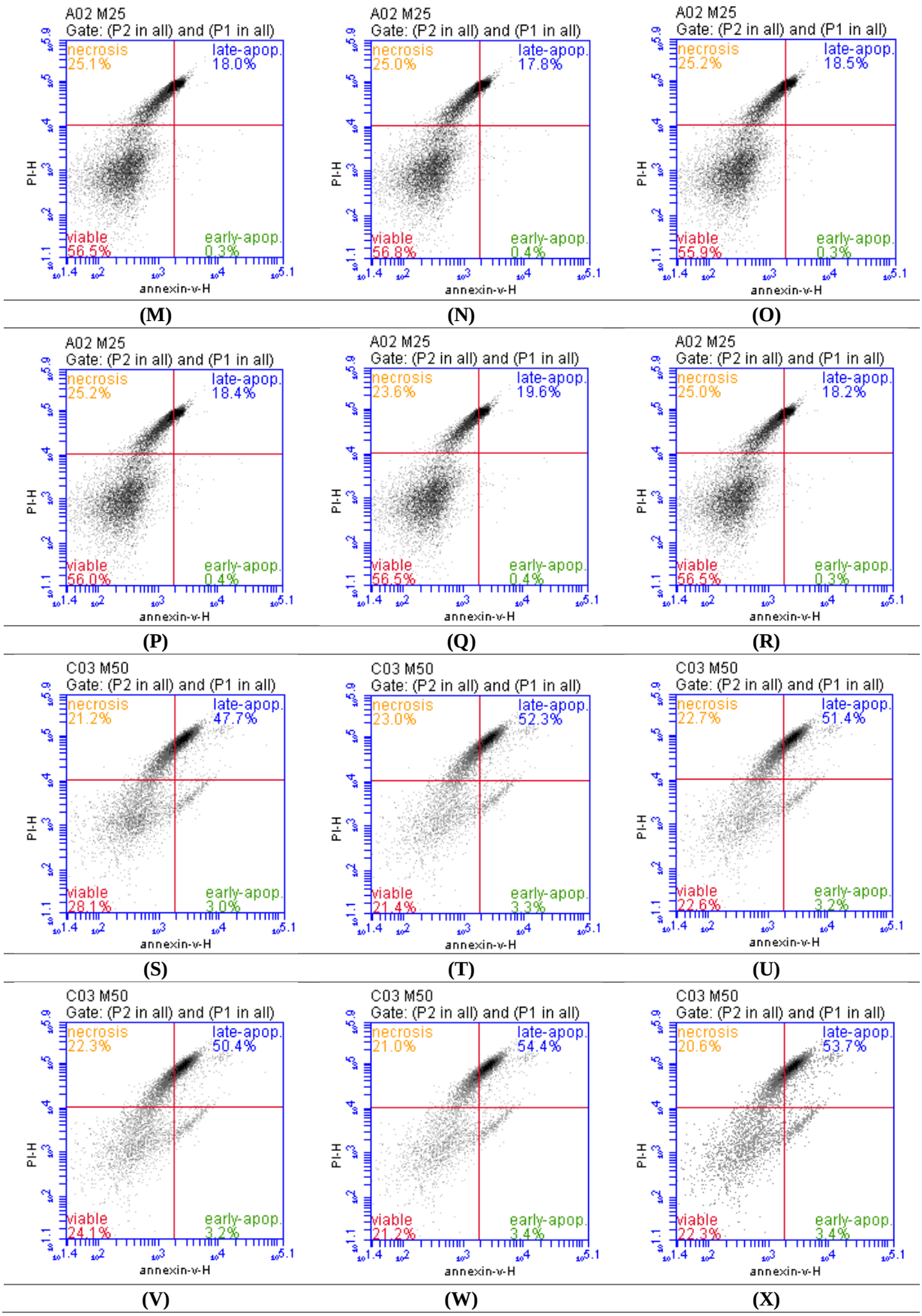



**Figure 5:** Flow cytometry analysis of apoptosis in MDA-MB-231 cells treated with Halfa Bar crude methanolic extract using Annexin V-FITC/7-AAD staining after 24 hours. Panels (A-X) display representative dot plots for different treatment groups. Panels A-B: Untreated control cells, predominantly viable (Annexin $V^-$/7-$AAD^-$). Panels C-H: $IC_{25}$-treated cells showing an increase in early apoptotic cells (Annexin $V^+$/7-$AAD^-$). Panels I-N: $IC_{50}$-treated cells exhibiting a marked rise in both early and late apoptotic populations (Annexin $V^+$/7-$AAD^+$). Panels O-X: Additional replicates or time-point variations illustrating dose- and time-dependent apoptosis. Each quadrant represents distinct cell populations: viable (lower left), early apoptotic (lower right), late apoptotic (upper right), and necrotic (upper left). Results confirm a concentration-dependent induction of apoptosis by Halfa Bar crude methanolic extract, aligning with cytotoxicity and wound healing assay findings.

Polyphenolics and flavonoids are naturally occurring secondary metabolites found abundantly in plants, known for their potent antioxidant, anti-inflammatory, and anticancer properties. Among their many biological activities, the ability to modulate apoptosis or programmed cell death is of particular importance in the context of cancer prevention and therapy. Apoptosis plays a crucial role in maintaining cellular homeostasis, and its dysregulation is a hallmark of cancer and other chronic diseases (Elmore, 2007). Several studies have established that polyphenolic compounds can induce apoptosis in cancer cells through a variety of molecular pathways, including the mitochondrial (intrinsic) and death receptor-mediated (extrinsic) mechanisms.

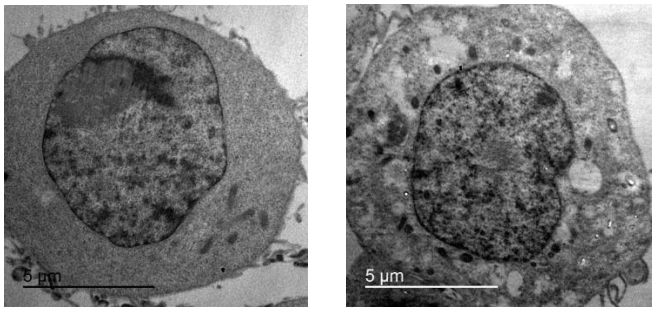
Polyphenols, such as epigallocatechin gallate (EGCG), curcumin, resveratrol, and quercetin, initiate apoptosis by generating reactive oxygen species (ROS), which lead to mitochondrial membrane depolarization and the subsequent release of cytochrome c into the cytosol. This triggers the activation of caspase-9 and caspase-3, key executioner enzymes in the intrinsic pathway (Khan *et al*., 2021). Concurrently, polyphenolics can upregulate pro-apoptotic proteins such as Bax and downregulate anti-apoptotic proteins like Bcl-2, tipping the balance toward cell death (Zhao *et al*., 2020). On the other hand, the extrinsic pathway is modulated through the activation of death receptors (e.g., Fas, TRAIL receptors) that play a crucial role in programmed cell death (apoptosis). Resulting in the recruitment of adaptor proteins and activation of caspase-8, which may directly cleave executioner caspases or amplify the apoptotic signal via the mitochondrial pathway (Wang *et al*., 2021).

Flavonoids a large subclass of polyphenols including flavonols, flavones, flavanones, and anthocyanidins also exhibit apoptosis-inducing effects. For instance, apigenin and luteolin have been shown to inhibit survival pathways such as PI3K/Akt and NF-κB, which are frequently upregulated in cancer cells to evade apoptosis (Mishra *et al*., 2020). Moreover, flavonoids can modulate p53 signaling, a key tumor suppressor pathway, leading to cell cycle arrest and apoptotic induction in malignantly transformed cells (Salehi *et al*., 2020). Beyond cancer, the pro-apoptotic activity of flavonoids is beneficial in controlling hyperproliferative disorders and in removing damaged or infected cells.

Modern techniques such as flow cytometry, western blotting, and gene expression profiling have validated these mechanisms, confirming that polyphenolic-rich extracts or purified compounds trigger caspase activation, DNA fragmentation, and other hallmarks of apoptosis (Khurana *et al*., 2021). Importantly, these compounds tend to exhibit selective cytotoxicity, preferentially inducing apoptosis in cancerous or damaged cells while sparing healthy ones an attribute that makes them highly attractive for integrative or adjunctive cancer therapies.

Transmission electron microscopy (TEM) provided ultrastructural evidence of apoptosis in cells treated with the Halfa Bar (*Cymbopogon proximus*) methanolic extract, in comparison to untreated control cells. Figure (6) represented the control group (Image A), cells exhibited normal ultrastructural morphology, characterized by intact plasma membranes, well-defined nuclei with uniformly dispersed chromatin, and abundant cytoplasmic organelles such as mitochondria and endoplasmic reticulum. These features are indicative of healthy, metabolically active cells and confirm the absence of spontaneous cell death under physiological conditions.

In contrast, cells treated with the Halfa Bar methanolic extract (Image B) displayed hallmark features of apoptosis. These included plasma membrane blebbing, nuclear condensation (pyknosis), margination of chromatin along the nuclear envelope, and cytoplasmic shrinkage. Additionally, mitochondrial swelling and the presence of apoptotic bodies membrane-bound cellular fragments were evident, strongly supporting the activation of programmed cell death pathways. These structural changes are consistent with the intrinsic (mitochondrial) apoptotic pathway, commonly induced by phytochemicals such as flavonoids and phenolic acids present in the extract (Wang *et al*., 2020).




**(A)** **(B)**

**Figure 6:** TEM of apoptosis in cells, (A) untreated control cell, and (B) treated cell with the Halfa Bar methanolic extract.

The observed morphological features align with earlier biochemical and flow cytometric data, including increased Annexin V binding and cytotoxicity revealed by the MTT assay. The extract's pro-apoptotic effects may be mediated through mitochondrial membrane depolarization, reactive oxygen species (ROS) generation, and caspase activation, as commonly reported for polyphenol-rich plant extracts (Kumar *et al*., 2018). These findings support the potential anticancer and cytotoxic applications of Halfa Bar extract, though its use must be carefully modulated to avoid undesired tissue damage.

In conclusion, polyphenolics and flavonoids represent a powerful class of bioactive compounds capable of modulating apoptosis through multiple interrelated molecular mechanisms. Their roles in targeting the redox balance, death receptor signaling, mitochondrial function, and transcriptional regulation position them as promising natural agents in the fight against cancer and other diseases characterized by apoptotic dysregulation.

## Wound healing potential of Halfa Bar methanolic extract

The wound healing assay, also known as the scratch assay, is a simple and widely used in vitro method to study cell migration and tissue regeneration, particularly in epithelial or fibroblast cell cultures. In this assay, a uniform "wound" or scratch is mechanically introduced into a confluent cell monolayer using a sterile pipette tip or similar tool. After scratching, the cells are incubated in serum-free or low-serum medium to minimize proliferation, allowing for the assessment of migration rather than cell division. The rate at which cells migrate to close the wound area is monitored over time, typically through time-lapse microscopy or image analysis, providing quantitative data on cell motility and

regeneration capacity. This assay is commonly used to evaluate the effects of bioactive compounds, growth factors, or genetic modifications on wound healing behavior (Liang *et al.*, 2021). Despite its simplicity and cost-effectiveness, the assay requires careful control of experimental variables to ensure reproducibility. According to Trinh *et al.*, (2022), wound healing is a process consisting of four phases: hemostasis, inflammation, proliferation, and remodeling. Illustration of the wound healing process is shown in Figure (7).

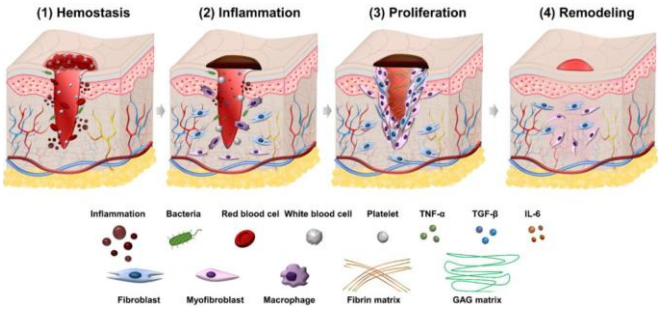



**Figure 7:** Illustration of four phases in the wound healing process.

The wound healing assay effectively demonstrated the migratory and proliferative capacity of cells under both untreated and Halfa Bar methanolic extract-treated conditions were represented in Figure (8). In the control group, cells exhibited a 37.10% wound closure over 24 hours, as the wound area reduced from 1,409,445.544 µm² to 886,566.491 µm². This natural closure reflects the intrinsic regenerative ability of healthy cells, consistent with previous findings on basal cell motility and monolayer repair under physiological conditions (Liang *et al.*, 2021). In contrast, treatment with the $IC_{25}$ concentration (~77.50 µg/mL) of Halfa Bar extract led to a negative wound closure percentage of -24.51%, where the wound area increased from 1,157,171.903 µm² to 1,440,775.096 µm². This inverse effect highlights a significant inhibition of cell migration and proliferation, likely due to early cytotoxic impact, as corroborated by MTT assay findings. Similarly, the $IC_{50}$ treatment (~155.13 µg/mL) produced an even more pronounced inhibitory effect, with a -48.42% closure, as the wound area increased from 1,027,704.851 µm² to 1,525,337.944 µm². These results suggest that the Halfa Bar methanolic extract, at cytotoxic concentrations, severely impairs wound healing by reducing cellular adhesion, viability, and motility, possibly through apoptosis or membrane integrity disruption. The dose-dependent inhibition observed is consistent with literature indicating that plant-derived cytotoxic compounds can suppress wound closure through antiproliferative and antimigratory mechanisms (Trinh *et al.*, 2022). Overall, the data support the notion that while Halfa Bar exhibits strong bioactivity, its application in wound healing must be cautiously dosed to avoid counterproductive cytotoxic effects. This finding confirms the intrinsic ability of healthy, proliferative cells to migrate and repopulate the damaged area in physiological conditions, as previously reported in basal cell locomotion and monolayer culture repair.

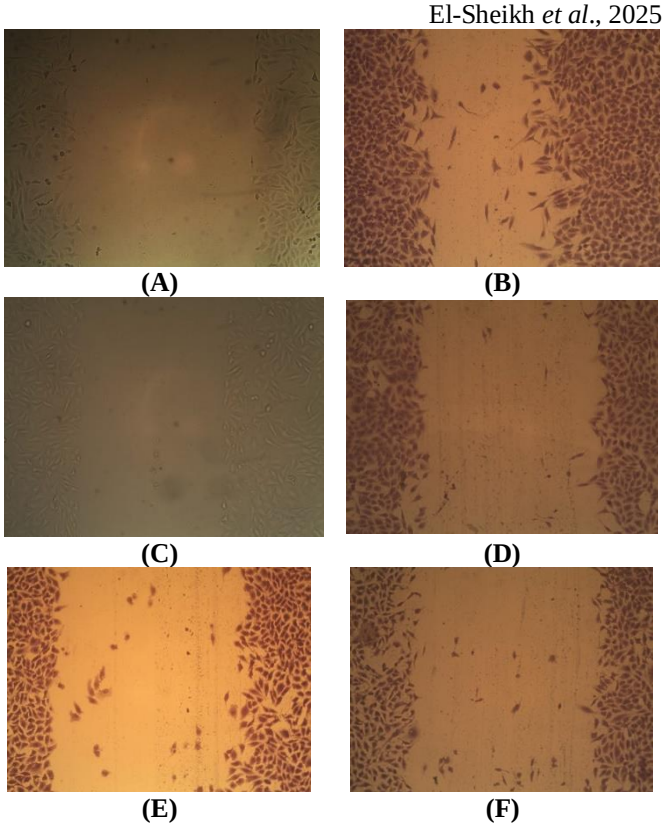



**Figure 8:** Effect of wound closure in MDA-MB-231 cells as observed in a scratch assay. (A and B) Representative images of the wound area at 0 time and after 24 hours in the untreated control group, respectively. (C and D) Representative images of the wound area at 0 time and after 24 hours in the treated cells with $IC_{25}$ dose of the Halfa Bar methanolic extract. (E and F) Representative images of the wound area at 0 time and after 24 hours in the treated cells with $IC_{50}$ dose of the Halfa Bar methanolic extract.

The inhibitory effects of the Halfa Bar (*Cymbopogon proximus*) methanolic extract on wound healing, particularly at $IC_{25}$ and $IC_{50}$ concentrations, are consistent with previous studies on the antiproliferative and antimigratory effects of polyphenol- and flavonoid-rich plant extracts. Several studies have reported that bioactive constituents such as flavonoids, tannins, and phenolic acids widely present in *C. proximus* exert cytotoxic and anti-migratory effects through the modulation of cellular redox balance, inhibition of survival signaling pathways, and induction of apoptosis (Arafa *et al.*, 2021 and Gendy *et al.*, 2021). For example, polyphenols such as catechins and gallic acid are known to interfere with the PI3K/Akt and MAPK/ERK pathways, which are critical regulators of cell migration, proliferation, and wound healing (Awasthi and Srivastava, 2024). Inhibiting these pathways results in reduced cell adhesion, cytoskeletal reorganization, and suppression of matrix metalloproteinases (MMPs), all of which are essential for cell migration during tissue regeneration (Yanagisawa *et al.*, 2022). Moreover, the increase in wound area observed at higher concentrations of the Halfa Bar extract may be attributed to a combination of apoptotic cell loss and disrupted actin polymerization at the wound edge. The cytotoxicity observed in the MTT assay supports this, indicating mitochondrial dysfunction and possible caspase activation, both hallmarks of programmed cell death. These findings are consistent with the known apoptotic mechanisms of plant phenolics, which often involve mitochondrial membrane depolarization and ROS

generation, ultimately leading to DNA fragmentation and inhibition of cell cycle progression (Wang *et al.*, 2020).

At therapeutic or sub-cytotoxic doses, *C. proximus* methanolic extract may support antioxidant defense and possibly aid in wound healing; however, at $IC_{25}$ and $IC_{50}$ concentrations, the high content of cytotoxic phytochemicals such as catechin, gallic acid, and chlorogenic acid likely initiates apoptotic pathways via: ROS generation leading to oxidative stress, Mitochondrial dysfunction and activation of intrinsic apoptosis (caspase-9 and caspase-3), Inhibition of pro-survival pathways such as PI3K/Akt and MAPK, Suppression of MMP activity and cytoskeletal remodeling, reducing cell motility, Disruption of cell adhesion and intercellular junctions, further exacerbating wound expansion. These mechanisms together impair both the proliferative and migratory capacities of cells, ultimately leading to impaired wound closure or even wound area expansion, as observed in this study.

## CONCLUSION

In conclusion, the findings of this research demonstrate that *Cymbopogon proximus* (Halfa Bar) extracts possess promising biological activities, particularly in cancer therapy, where methanolic and ethyl acetate extracts showed moderate cytotoxicity against MDA-MB-231 breast cancer cells through dose-dependent induction of apoptosis, confirmed by flow cytometry and ultrastructural analysis. These effects are likely mediated by the plant's rich content of polyphenols and flavonoids, which trigger oxidative stress, mitochondrial dysfunction, and caspase activation. However, while the extracts exhibited strong anticancer and pro-apoptotic potential, their application in wound healing was limited, as higher cytotoxic concentrations impaired cell migration and wound closure in vitro. Collectively, these results validate the traditional medicinal use of *C. proximus* and highlight its potential as a natural source of anticancer agents, though further studies are needed to optimize dosage and evaluate its safety and therapeutic efficacy in vivo.

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