Natural Resources for Human Health

visagaa publishing house

Original Research

View Article online

Check for updates

Received 28 March 2026
Revised 05 May 2026
Accepted 16 June 2026
Available Online 28 August 2026

Edited by Prathapan Ayyappan

KEYWORDS:
BCL2
BAX
Apoptosis
Antioxidant activity
HeLa cell line
Pogostemon parviflorus

Natr Resour Human Health 2026; 6 (4): 542–551
https://doi.org/10.53365/nrfhh/224639
eISSN: 2583-1194

Pogostemon parviflorus promoting apoptosis of cervical cancer cell line: an *in vitro* study

Harsha Thanvi[1], Jayshree Changade[1,*], Vaibhav Sunil Ladke[2]

[1]Department of Dravyaguna Vigyana, Dr. D. Y. Patil College of Ayurved & Research Centre, Dr. D. Y. Patil Vidyapeeth (Deemed to Be University). Sant Tukaram Nagar, Pimpri, Pune, India
[2]Oncology Research, Integrative Health From Research to Complete Care, Pune India

ABSTRACT: Cervical cancer is prevalent among women and is associated with a significant mortality rate. Chemotherapy is frequently employed as a treatment modality; however, its efficacy in addressing cervical cancer is constrained by significant side effects and the development of treatment resistance. The research evaluates the anticancer properties of *Pogostemon parviflorus* extract on a human cervical cancer cell line. The hydroalcoholic extract of the leaf portion of *P. parviflorus* was meticulously prepared employing established methodologies. The research investigated the anticancer properties of the plant using various *in vitro* methodologies, including 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide assays, apoptosis evaluation, cell cycle analysis, assessment of reactive oxygen species, mitochondrial membrane potential analysis, and RT-PCR for gene expression profiling. The findings indicated a pronounced cytotoxic effect, characterized by early apoptosis and disturbances in the sub-G0 phase of the cell cycle. The presence of *P. parviflorus* resulted in a significant reduction of reactive oxygen species within cells. The analysis of gene expression revealed significant effects on the BAX, BCL-2, and cMYC genes, indicating the onset of early apoptosis and demonstrating antiproliferative effects. This study reveals that *P. parviflorus* may serve as a promising candidate for the treatment of cervical cancer, owing to its cytotoxic, antiproliferative, apoptotic, and antioxidant characteristics. Additional investigation is necessary to elucidate its mechanism and substantiate its viability as a prospective cancer therapy.

1. INTRODUCTION

Cervical cancer continues to be a significant global issue, with cervical cancer being the fourth most prevalent cancer among women, following breast, colorectal, and lung cancers. Based on GLOBOCAN 2022, it is anticipated that there were approximately 662,301 new cases of cervical cancer worldwide in 2022, resulting in approximately 348,874 fatalities annually (Bray et al., 2024). In low- and middle-income countries, this malignancy is the third most prevalent among women, with a significant proportion of new cases and fatalities, approximately 85% and 90%, respectively. In 2022, it was anticipated that 1,461,427 new cases of cancer would be diagnosed in India, reflecting a cumulative rate of 100.4 cases per 100,000 individuals, as per the research conducted by Sathishkumar et al. The research suggests that cancer may develop in one in nine Indians at some point in their lives, which is concerning. Lung cancer was the most prevalent form of cancer among men, while breast cancer was the most prevalent form of cancer among women. The information obtained from these estimates can be of great assistance in the creation of targeted programs that are designed to prevent

* Corresponding author.
E-mail address: jayshreeulemale28@gmail.com (Jayshree Changade)

and control cancer. These endeavors may encompass effective management techniques, risk factor mitigation strategies, and early detection programs (Sathishkumar et al., 2022).

Thorough investigations aim to develop more effective therapies that reduce adverse effects. Focus is on creating therapies that specifically target and eliminate malignant tumor cells while leaving normal cells unharmed. This effort aligns with the goal of achieving cancer chemotherapy, where targeted drugs can destroy cancerous cells or help convert them into non-cancerous forms (Sporn and Liby, 2005). Addressing this challenge requires examining traditional herbs as viable options for drug development. Traditional medicine has relied heavily on plant resources, which have played a key role in its advancement.

Numerous phytochemicals and their metabolites have a wide spectrum of pharmacological actions in the human body. This includes flavonoids, phenolics, alkaloids, tannins, gums, oils, glycosides, and resins (Ladke et al., 2022). The bioactive components of these plant-derived drugs are largely responsible for their diverse therapeutic effects (Buva et al., 2025; Kumbhar et al., 2024; Sinnarkar et al., 2024; Singh et al., 2016). Due to its availability in a wide range of retail establishments in addition to pharmacies, herbal medicines offer a special advantage in terms of consumer accessibility. Approximately 80% of the world's population, mostly from Asian and African nations rely on herbal therapy (Chandra et al., 2013). Many of the medications that are vital to modern medicine have their origins in plants (Newman and Cragg, 2016).

The Lamiaceae family's genus *Pogostemon* is well known for its several pharmacological and ethnomedical uses (Momin et al., 2026). There is still a significant knowledge gap regarding *P. parviflorus*, despite the substantial research conducted on related species like *P. cablin* and *P. benghalensis*, which have identified a variety of phytochemicals with antibacterial and anti-inflammatory qualities, including terpenoids, flavonoids, and glycosides (Dahiya et al., 2020; Singh and Agrawal, 2024). The fragrant, woody herbaceous plant Phanijjaka (*P. parviflorus,* Benth) is a member of the Lamiaceae family and is commonly found in areas of India with significant rainfall (Desai, 1975). This plant is used to treat fever, colic, eczema, enteritis, and vaginitis because of its antibacterial qualities. This plant's roots have been used to treat uterine bleeding (Bhandari, 1993). Because of its anticancer qualities, *P. benghalensis* has long been used in India. Ayurvedic practitioners treat many types of cancer with the Phanijjaka plant (*P. parviflorus*, Benth). Reports on this plant's *in vitro* activity in gynaecological cancer cell lines are currently lacking. Therefore, the current study assessed *P. parviflorus's* anticancer potential in relation to cervical cancer using a range of parameters relevant to carcinogenesis.

2. MATERIALS AND METHODOLOGY

2.1. Plant material

The aerial parts, specifically the leaves, of *P. parviflorus* were sourced from the Vidarbha region of Maharashtra, India. The Botany Department of the Agharkar Research Institute in Maharashtra, India, conducted the verification of the authenticity and precise identification of the plant samples under the identification code AUTH20-138.

2.2. Extract preparation

The newly harvested leaves of *P. parviflorus* were meticulously cleansed and subsequently crushed. The hydroalcoholic extract was obtained by subjecting 50 g of freshly crushed leaves to Soxhlet extraction using a mixture of ethanol and distilled water in a ratio of 70:30 at a temperature of 45°C. Following filtration with Whatman filter paper, the substance was either dried or vaporised in a water bath maintained at 45°C and subsequently sealed within an airtight container.

2.3. Preparation of stock solution of extract

A stock solution with a concentration of 10 mg/mL was prepared by diluting a 10 mg hydroalcoholic extract of PP in 1 mL of dimethyl sulfoxide (DMSO) and thereafter stored at −80°C. A stock solution with a concentration of 10 mg/mL was utilized to prepare a 1 mg/mL extract in a complete medium. The solution was filtered using aseptic 0.22 µm filters and subsequently used to prepare dilutions. A comprehensive review of the literature and the use of logarithmic concentrations led to the preparation of formulations at 20, 40, 80, 100, 150, 200, and 250 µg/mL in a full culture medium to evaluate their cytotoxic effects on the HeLa cell line. The maximum concentration of DMSO employed in the studies was below 0.1%.

2.4. Cell line and culture maintenance

The HeLa cancer cell line, derived from human cervical carcinoma, was procured from the National Centre for Cell Science (NCCS) in Pune, India. The cells were grown in Minimum Essential Medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), L-glutamine, and 1% antibiotics, comprising 10,000 units of penicillin, 10 mg of streptomycin, and 25 µg of amphotericin B per milliliter.

The cells were cultivated at 37°C in a humidified atmosphere supplemented with 5% CO_2.

2.5. Cell cytotoxicity assay

The evaluation of cellular cytotoxicity was performed using the 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) conversion assay (Mosmann, 1983). Hela cells were cultured at a density of 5 × 10^3 cells/mL on a 96-well plate. PP was employed at doses of 20, 40, 80, 100, 150, 200, and 250 µg/mL. Subsequent to the treatment, 20 µL of a 5 mg/mL MTT solution was introduced to each well and permitted to incubate for an additional 4 h. To promote the disintegration of the formazan crystals in each well, 100µL of DMSO was added. The evaluation of purple formazan synthesis in correlation with viable cell counts was performed by measuring absorbance at 570 nm using a MultiscanGo Thermofisher ELISA plate reader. The IC_{50} value was determined using a conventional algorithm in a Microsoft Excel spreadsheet.

2.6. Apoptosis using Annexin V

The FITC Annexin V/Dead Cell Apoptosis Kit from Invitrogen-Molecular Probes® was utilized for the apoptosis assay. After a 24-h treatment period, HeLa cells were subjected to a cold wash with 1× Phosphate-buffered saline (PBS). The untreated cells served as the control group. The assay strictly followed the manufacturer's protocol. Flow cytometry was utilized to evaluate labelled cells, focusing on emission wavelengths of 530 nm and above 575 nm, thereby enabling the assessment of apoptosis in treated cells relative to controls (Changade et al., 2024; Kanase et al., 2025; Wyreogonekbska et al., 2013).

2.7. Cell cycle analysis

After rinsing with 1× PBS and subsequent trypsinization, HeLa cells treated with *P. parviflorus*, as well as their untreated counterparts, were collected after a 24-h period. Subsequently, the harvested cells were subjected to treatment with 25 µl of RNase A (20 mg/mL from Invitrogen), 2 mM $MgCl_2$ (from Sigma), and 5–10 µL of propidium iodide at a concentration of 100 µg/mL (from Invitrogen). The cells were then cultured for an additional 10–15 minutes at room temperature before being analyzed using the FACS Calibre from BD Bioscience. This approach facilitated a comprehensive analysis of the cells' properties via flow cytometry, intended to clarify their cellular makeup and state (Changade et al., 2024; Kanase et al., 2025).

2.8. Determination of intracellular ROS generation

Flow cytometry was utilized to examine ROS generation using DCFHDA (Brandt and Keston, 1965). HELA cells were cultured in six-well plates for 24 h for this test. Cells were treated with *P. parviflorus* at doses equivalent to the IC_{50} and IC_{25} values, in conjunction with 10 µM DCFH-DA. Untreated cells were incubated with 10 µM DCFH-DA, and the maximum dosage of DMSO was used. Flow cytometry (BD Bioscience) was utilized to evaluate the fluorescence generated from the hydrolysis of DCFH-DA to dichlorodihydrofluorescein (DCFH) (Changade et al., 2024; Kanase et al., 2025; Wyreogonekbska et al., 2013).

2.9. Estimation of mitochondrial membrane potential (ΔΨm)

The mitochondrial membrane potential in HeLa cells was assessed using the MitoProbeTM DiIC1 (5) Assay Kit following the manufacturer's guidelines. The MitoProbeTM DiIC1(5) Assay Kit is designed to investigate ΔΨm and includes solutions of DiIC1(5), a cyanine dye sensitive to membrane potential, and CCCP, a chemical that interferes with mitochondrial membrane potential. DiIC1(5) permeates the cytoplasm of eukaryotic cells and mostly accumulates in mitochondria with active membrane potentials, leading to the emission of intense, far-red fluorescence. The intensity of DiIC1(5) staining lowers as mitochondrial membrane potential declines (Kumbhar et al., 2025).

2.10. RNA isolation and RT-PCR

Cells were grown at a density of 5 × 10^4 in 24-well plates and permitted to proliferate for 24 h prior to the commencement of treatment. The cells were then cultured for a period of 4 to 24 h in a new medium enriched with FBS and a combination of antimycotic and antibiotic agents. During this era, they faced various drug concentrations. Following drug incubation, cells were carefully collected, and total RNA was extracted using TRIzol™ Reagent (Invitrogen, cat no. 15596018), according to the manufacturer's instructions. The extracted RNA was submitted to reverse transcription for downstream analysis using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, cat no. 4368814) following the kit's manual instructions. The resulting cDNA samples were stored at –20°C for future use (Changade et al., 2024;

Table 1
Genes and their primers.

S.no.	Gene name	Forward	Reverse
1	BAX	5'-TCAGGATGCGTCCACCAAGAAG-3'	5'-TGTGTCCACGGCGGCAATCATC-3'
2	cMYC	5'-AGAAATGTCCTGAGCAATCACC-3'	5'-AAGGTTGTGAGGTTGCATTTGA-3'
3	BCL2	5'-TTGCTTTACGTGGCCTGTTTC-3'	5'-GAAGACCCTGAAGGACAGCCAT-3'

Kanase et al., 2025; Kumbhar et al., 2025). Every gene mRNA levels were standardized using β-actin as an endogenous reference, and relative measurement was performed using the $2^{-\Delta\Delta Ct}$ method. The primers utilized in this work are enumerated in Table 1.

2.11. Statistical analysis

All experiments were performed in triplicate and independently replicated three times. Statistical analyses were performed using GraphPad Prism (version 8.0.2). Results were expressed as mean ± standard error of the mean (SEM) and mean ± standard deviation (SD). Group differences were evaluated using one-way ANOVA, followed by Tukey's post hoc test. A p-value below 0.05 was considered statistically significant.

3. RESULTS

3.1. Cytotoxic activity of *P. parviflorus on* HeLa cell line

Assessments were performed on the HeLa cancer cell line utilizing hydroalcoholic (HA) extract of PP at varying concentrations of 20, 40, 80, 100, 150, 200, and 250 µg/mL. The PP hydroalcoholic extract exhibits a cytotoxic effect on the HeLa cell line that is dependent on dosage, with an IC_{50} value of 186 ± 13.20 µg/mL and an IC_{25} value of 93 ± 6.60 µg/mL (Figure 1A). HeLa cell viability decreased concentration-dependently with DMSO. Low doses (0.75 µg/mL) resulted in 95% cell viability, indicating minimal cytotoxicity. Low doses (1–2.5 µg/mL) caused an 84–86% drop in viability, indicating minor cellular stress without a dose-dependent response higher dosages (3–3.5 µg/mL) caused a significant reduction in viability (~74–79%), indicating increased cytotoxicity. Given its low toxicity, DMSO can be utilized as a solvent control at lower doses since cell survival was above 70% at all dosages (Figure 1B).

3.2. *P. parviflorus* affecting HeLa cell morphology

After administering *P. parviflorus* to HeLa cells at IC_{50} and IC_{25} doses over 24 h, notable alterations in cell shape were observed. Post-treatment with PP, the HeLa cells exhibited an atypical structural alteration, characterized by a rounded and irregular shape. It is noteworthy that the ability of the HeLa cell line to adhere was diminished following the treatment with PP (Figure 2).

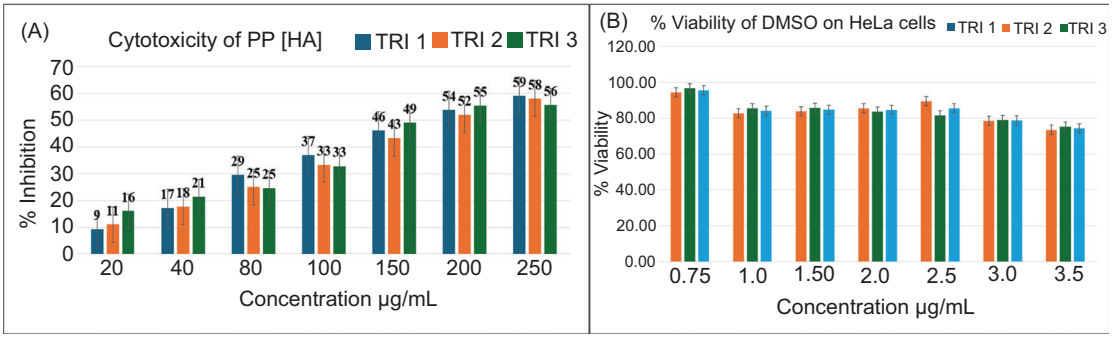

Figure 1. (A) Percent inhibition of *Pogostemon parviflorus* on HeLa cell line at different concentrations. *X*-axis denotes various concentrations of PP and *Y*-axis indicates % inhibition of HeLa cells (n = 3). (B) Percent viability of DMSO on HeLa cell line at different concentrations. *X*-axis denotes various concentrations of DMSO, and *Y*-axis indicates % viability of HeLa cells (n = 3). (TRI = triplicate).

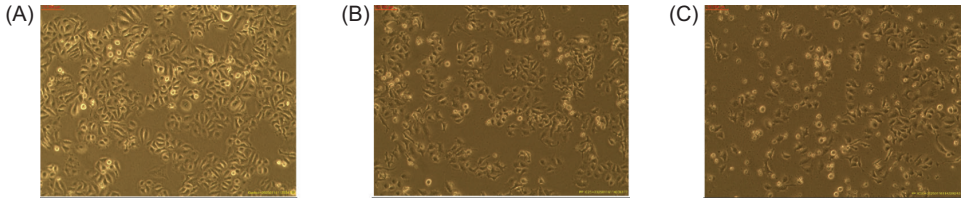

Figure 2. Morphological changes observed after PP treatment on HeLa cells. (A) Control: HeLa cells have a normal polygonal shape. (B) PP IC_{25} treatment: HeLa cells reveal a round to slender shape. (C) PP IC_{50} treatment: floating cells that range from irregular to round in shape. (Magnification at 200×).

3.3. *P. parviflorus* promoting apoptosis on HeLa cells

The results demonstrated that exposure to the IC_{50} concentration of PP resulted in 53.20 ± 2.13% of cells progressing into the early phase of apoptosis, whereas 7.78 ± 4.2% of cells were observed in the late phase of apoptosis. The IC_{25} concentration of PP exhibited a significant 36.80 ± 1.93% of cells undergoing early apoptosis, in conjunction with 4.49 ± 3.10% of cells in the late phase of apoptosis (Figure 3).

3.4. *P. parviflorus* modulating cell cycle regulation of HeLa cells

The results indicated a notable rise in cell accumulation in the Sub-G0/G1 phase following the therapy. The Sub-G0 population increased to 67.7 ± 2.10% at IC_{25} and further to 91.4 ± 1.59% at IC_{50}, relative to the untreated control cells. The Sub-G0 phase denotes cells with fragmented DNA and reduced DNA content, a hallmark of late apoptosis. Thus, the substantial rise in the Sub-G0 population evidently indicates the initiation of apoptosis rather than a mere halt in the cell cycle. The observed rise across several concentrations from IC_{25} to IC_{50} demonstrates that higher doses of PP extract amplify apoptotic signaling, leading to substantial DNA breakage and ensuing cell death. The substantial accumulation (surpassing 90%) of cells in Sub-G0 at IC_{50} signifies a notable level of cytotoxic and pro-apoptotic action. This trend indicates that the extract does not merely impede cell cycle progression at essential checkpoints such as G_0/G_1, S, or G_2/M phases. Instead, it advances cells beyond a condition of stagnation towards the initiation of apoptosis, so effectively suppressing cellular proliferation. The findings demonstrate that PP possesses significant antiproliferative effects on cervical cancer (HeLa) cells by inducing apoptosis, as seen by a marked increase in the Sub-G0 phase in a dose-dependent pattern (Figure 4).

3.5. Impact of *P. parviflorus* on intracellular ROS measurement

Figure 5 shows that administering PP to HeLa cells at IC_{25} (geometric mean = 1293± 11.38) and IC_{50} (geometric mean = 1573 ± 09.27) doses for 24 h significantly reduced intracellular reactive oxygen species (ROS) levels compared to untreated control cells (geometric mean = 7792 ± 13.21), which had 74.23 ± 1.38% ROS-positive cells. Both amounts

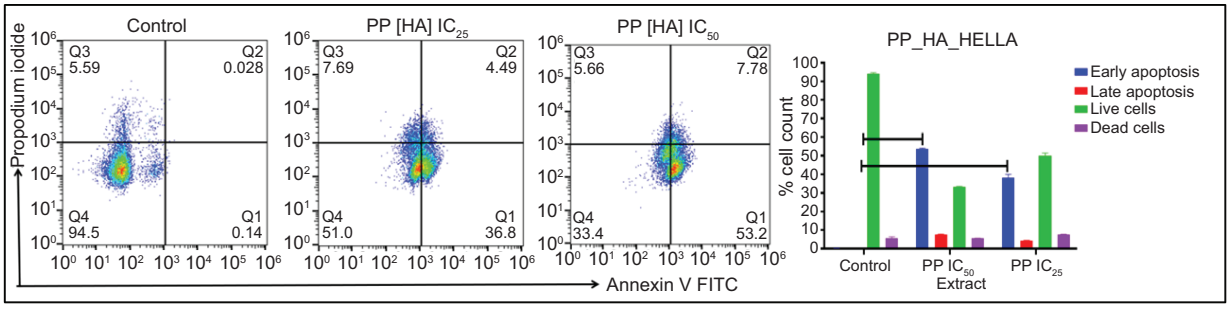

Figure 3. The impact of PP on apoptosis in HeLa cells during treatment. The histogram illustrates the apoptosis ratios of HeLa cells under control conditions and following treatment with PP at IC_{25} and IC_{50} concentrations over a 24-h period, utilizing propidium iodide and Annexin-V FITC for analysis. The bar graph illustrates the proportions of dead, live, early apoptotic, and late apoptotic cells in both the control group and those treated with PP at IC_{25} and IC_{50} concentrations over a 24-h period. Data are expressed as mean ± SD (n = 3). ****P < 0.0001 versus the control group (untreated cells).

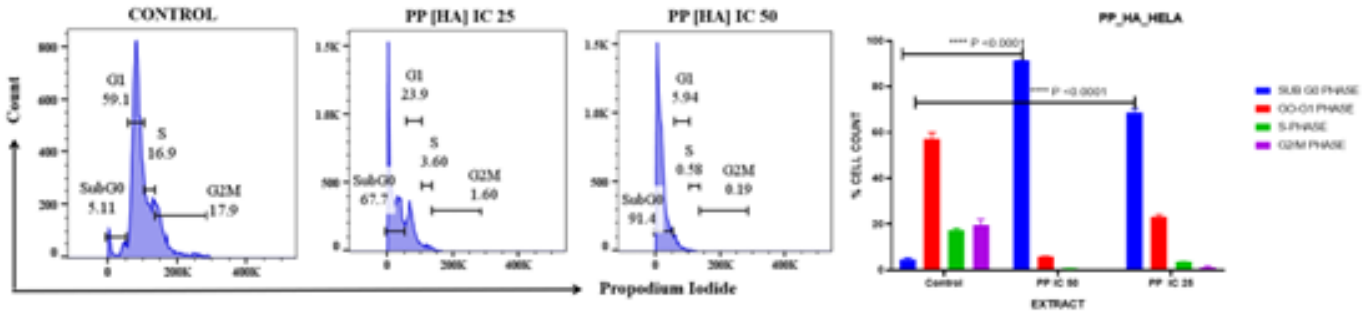

Figure 4. The impact of PP on the cell cycle dynamics in HeLa cells. A histogram illustrates the various phases of the cell cycle in HeLa cells, both in a control state and following treatment with PP at IC_{25} and IC_{50} concentrations over a 24-h period, utilizing propidium iodide for analysis. The bar graph illustrates the proportions of cells in the sub-G0, G1, S, and G2/M phases, both in the control group and following a 24-h treatment with PP. Data are expressed as mean ± SD (n = 3). ****P < 0.0001 versus the control group (untreated cells).

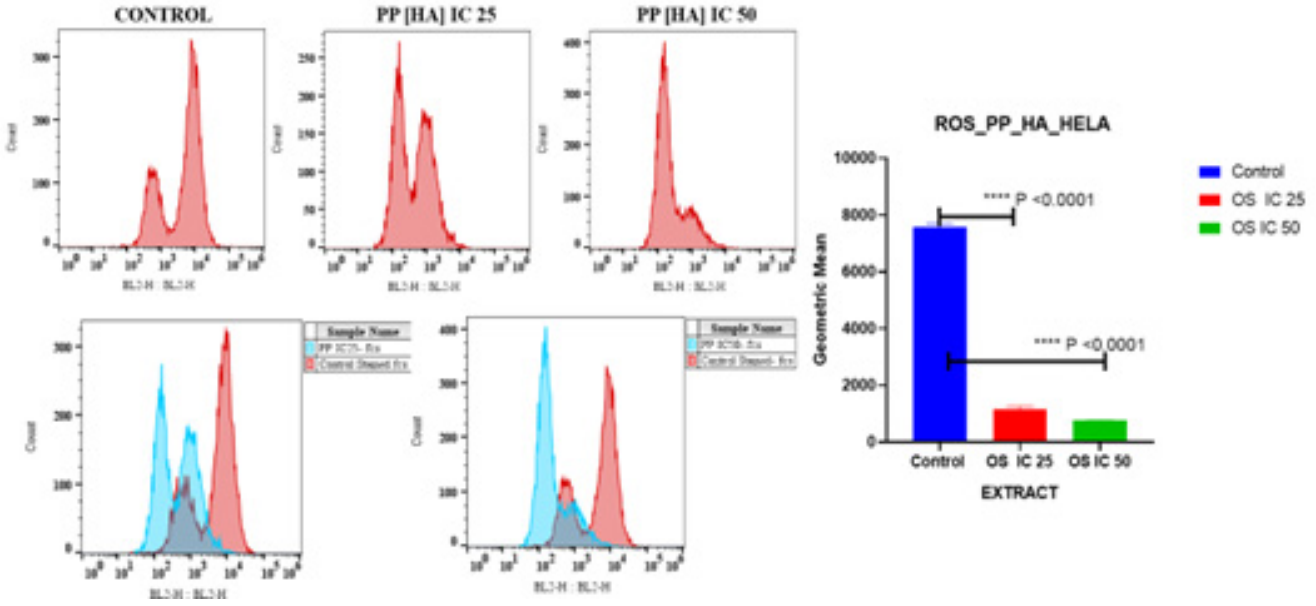

Figure 5. Impact of *Pogostemon parviflorus* on the generation of intracellular reactive oxygen species. The flow cytometry histogram illustrates the degree of reactive oxygen species (ROS) production prior to (blue) and subsequent to the treatment of PP with concentrations of IC_{25} (red) and IC_{50} (green) over a duration of 24 h. The bar graph illustrates the quantitative assessment of ROS production prior to treatment (blue) and following the application of *Pogostemon parviflorus* at IC_{25} (red) and IC_{50} (green) concentrations over a duration of 24 h. Data are expressed as mean ± SD (n = 3). ****P < 0.0001 versus the control group (untreated cells).

of PP significantly lowered ROS production, showing a concentration-dependent antioxidant or ROS-modulating effect. The decrease at IC_{50} was more significant, showing that greater exposure enhances the compound's capacity to modulate oxidative stress in cells. Increased ROS are often linked to apoptosis, yet certain anticancer treatments can induce programmed cell death through mitochondrial mechanisms that do not rely on ROS. These findings highlight the significant impact of PP on intracellular redox status and suggest its potential role in regulating oxidative stress in HeLa cells in a dose-dependent manner.

3.6. Effect of *P. parviflorus* in alteration in mitochondrial membrane potential [ΔΨm]

Figure 6 demonstrates that the administration of PP to HeLa cells at both IC_{50} and IC_{25} doses resulted in a

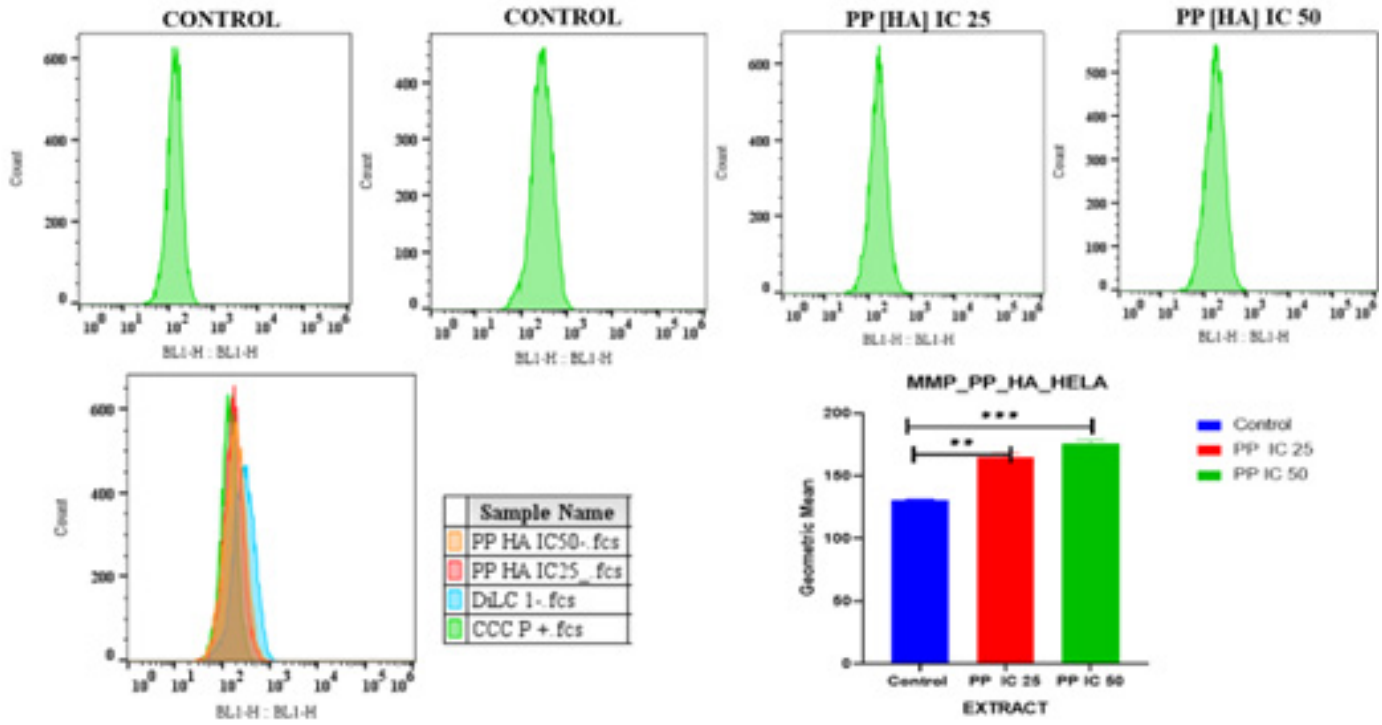

Figure 6. Analysis of mitochondrial membrane potential through flow cytometry. The histogram illustrates the effects on cells subjected to PP at both IC_{50} and IC_{25} concentrations following a 24-h treatment period. Data are expressed as mean ± SD (n = 3). ***P < 0.001 and **P < 0.01 versus the control group (untreated cells).

significant alteration in ΔΨm compared to control cells. Cells treated at IC_{50} had a statistically significant effect (***P < 0.001) relative to IC_{25}, indicating a more substantial impact at the higher dose. The observed change in ΔΨm signifies a depolarisation of the mitochondrial membrane, suggesting a compromise in mitochondrial integrity. This depolarisation is typically associated with increased mitochondrial permeability, the release of proapoptotic chemicals into the cytosol, and the activation of the intrinsic apoptotic pathway. The results demonstrate that PP promotes a dose-dependent impairment of mitochondria in HeLa cells, hence confirming its role in the start of mitochondria-mediated apoptosis.

3.7. Effects of *P. parviflorus* on BAX, BCL-2, and cMYC expression in HeLa cells

The hydroalcoholic extract of *P. parviflorus* (PP HA) affects critical regulators of apoptosis and proliferation in HeLa cells, particularly at the IC_{25} concentration, as indicated by gene expression analysis. The expression of BCL-2 markedly diminished following therapy. BCL-2 functions as an antiapoptotic protein that preserves mitochondrial membrane integrity and inhibits cytochrome C release. Decreased expression of BCL-2 impairs cellular survival signaling and heightens susceptibility to apoptosis. The notable decrease at IC_{25} suggests that diminished concentrations of PP HA can interfere with prosurvival processes. The extract decreased the expression of cMYC, a proto-oncogene that facilitates cell proliferation, metabolic activity, and cell cycle advancement. Reduced cMYC expression signifies decreased proliferative signaling, underscoring the extract's antiproliferative capability. This corroborates previous results of cell cycle disruption and reduced cell viability. PP HA extract stimulated the overexpression of BAX, a pro-apoptotic member of the BCL-2 family. BAX facilitates the permeabilization of the mitochondrial outer membrane, allowing for the release of cytochrome C and initiating the intrinsic apoptotic pathway. The increase in BAX and decrease in BCL-2 modify the BAX/BCL-2 ratio to facilitate apoptosis, a crucial element in mitochondrial-mediated cell death. The simultaneous downregulation of BCL-2 and cMYC, coupled with the overexpression of BAX, indicates that PP HA extract mostly facilitates apoptosis via the intrinsic (mitochondrial) apoptotic route while concurrently inhibiting proliferative signaling. Molecular alterations corroborate the documented apoptosis and antiproliferative effects in HeLa cervical cancer cells (Figure 7).

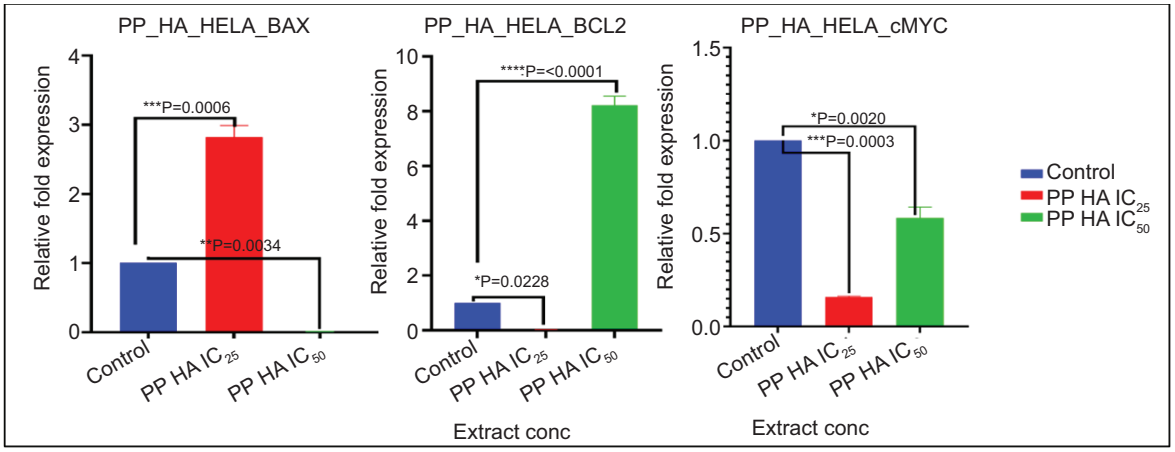

Figure 7. Investigation of the dose-dependent effects of PP on the expression of genes associated with apoptosis and proliferation in HeLa cells. The bar graph illustrates the alterations in gene expression levels of BAX, BCL-2, and cMYC, contrasting the states prior to (Blue) and subsequent to treatment with PP at concentrations of IC_{25} (red) and IC_{50} (green) over a duration of 24 h. Data are expressed as mean ± SD (n = 3). **P < 0.01, ***P < 0.001, ****P < 0.0001 versus the control group (untreated cells).

4. DISCUSSION

The growing interest in medicinal and aromatic plants (MAPs) can be attributed to their abundant bioactive compounds that enhance biological activity both *in vitro* and *in vivo*, leading to their rising prominence across various sectors, including industry, education, agriculture, and health. The secondary metabolites of MAP demonstrate superior efficacy compared to numerous synthetic and natural antioxidants, owing to their remarkable properties, including antioxidant, anti-inflammatory, antibacterial, antiviral, and anticancer activities. The observed characteristics can be attributed to a variety of compounds, including phenols, flavanols/flavonoids, alkaloids, polypeptides, vitamins, catechins, phytoestrogens, carotenoids, chlorophyll, and minerals (Chrysargyris, 2024).

The Lamiaceae family, known for its antioxidant and antibacterial properties, stands as a significant contributor to the production of essential oils. Aromatic plants are replete with essential oils. Numerous studies suggest that the inclusion of flavonoids in one's diet may lead to a decrease in the occurrence of pancreatic, breast, and colon cancers. Pharmacological agents that impede the cell cycle or induce apoptosis in malignant cells are employed in their treatment. The role of essential oils in the prevention and suppression of cancer is noteworthy. Numerous plants yield essential oils that have demonstrated efficacy against various forms of cancer, including those affecting the mouth, breast, lung, prostate, liver, colon, brain, and leukemia (Nieto, 2017).

The investigation into the effects of hydroethanolic and aqueous extracts of *P. benghalensis* on mice afflicted with Ehrlich ascites carcinoma (EAC) tumors revealed noteworthy anticancer activity in Swiss albino mice. The median survival time (MST) of tumor-bearing mice exhibited a notable enhancement due to the administration of hydroethanolic extracts (HEEPB), aqueous extracts (AEPB), and 5-Fluorouracil (5-FU) when juxtaposed with the tumor control group. Following a month of meticulous observation, the mice administered 5-FU (20 mg/kg), HEEPB (500 mg/kg), and AEPB (500 mg/kg) exhibited tumor volumes of 1.90, 1.67, and 1.62 mL, respectively, in contrast to the tumor control group's volume of 3 mL (Patel et al., 2014).

Elevated levels of reactive oxygen species in neoplastic cells lead to cellular instability and resistance to therapeutic interventions. Consequently, diminishing reactive oxygen species could enhance cellular stability and sensitivity to chemotherapeutic agents. Confirming this necessitates a thorough examination. This research substantiates the longstanding application of this plant in the context of cancer treatment. This represents the inaugural investigation conducted on the species *P. parviflorus* in relation to cervical cancer.

Currently, *P. parviflorus* exhibited cytotoxic effects on HeLa cells primarily through an early apoptotic mechanism at an IC_{25} concentration. PP demonstrated cell cycle arrest at the SubG0 phase once more, suggesting the occurrence of apoptosis. A notable decrease in intracellular reactive oxygen species indicates the antioxidant properties of PP. To evaluate the apoptotic and antiproliferative activity, specific genes were examined to elucidate the underlying mechanism. The findings indicated that PP enhanced the expression of BAX while simultaneously reducing the levels of BCL2 and cMYC genes, implying its potential apoptotic and antiproliferative characteristics. Thus, the current investigation elucidated the anticancer properties of PP through the promotion of apoptosis,

enhancement of antioxidant activity, and the suppression of cancer cell proliferation. It is evident that further *in vivo* investigations employing a variety of tumor models are essential for advancing our understanding. It is imperative to address the safety risks associated with *P. parviflorus* before proceeding with the clinical trials.

5. LIMITATIONS OF THE STUDY

The restricted availability and accessibility of validated normal cell lines within our laboratory framework in India during the study period hindered the assessment of the extract on noncancerous [cervical] cell lines from being conducted in this study. This represented a pragmatic constraint that precluded the inclusion of comparative cytotoxicity analysis. Moreover, *in vivo* studies were not conducted to ascertain therapeutic relevance, and the validation of apoptotic markers at the protein level (such as caspase-3) was also not performed to corroborate gene expression data. The aforementioned limitations do not diminish the proapoptotic activity observed in *P. parviflorus*; rather, they underscore the necessity for further investigation utilizing normal cell models, protein-level analyses, phytochemical standardization, and *in vivo* validation to comprehensively ascertain its therapeutic potential.

6. CONCLUSION

The findings of our investigation underscore the promise of *P. parviflorus* as a noteworthy source of active compounds exhibiting efficacy against cervical cancer cells. It is posited that these compounds play a substantial role in the manifestation of its therapeutic effects. Exploring the intricate molecular mechanisms underlying its action may be crucial for the development of future dosage formulations. In light of these findings, *P. parviflorus* presents an intriguing avenue for further investigation as a potential anticancer agent. Additional investigation and scholarly inquiry are essential to fully elucidate its mechanism of action and to advance it as a plausible option for cancer therapy.

ACKNOWLEDGMENTS

None.

AUTHOR CONTRIBUTION

Harsha Thanvi: Methodology, Data curation, Formal analysis, Methodology. Jayshree Changade: Conceptualization, Software, Formal analysis, Writing—original draft. Vaibhav Ladke: Formal analysis, Software, Investigation, Validation, Writing original draft. All authors approved the submitted manuscript.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

FUNDING

This study was not supported by any sponsor or funder.

ORCID

Harsha Thanvi 0000-0002-6536-6455
Jayshree Changade 0000-0001-7409-9419
Vaibhav Sunil Ladke 0000-0002-6327-3378

REFERENCES

Brandt, R., & Keston, A.S. (1965). Synthesis of diacetyldichlorofluorescin: a stable reagent for fluorometric analysis. Analytical Biochemistry, 11(1), 6–9. https://doi.org/10.1016/0003-2697(65)90035-7

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R.L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 74(3), 229–263. https://doi.org/10.3322/CAAC.21834

Buva, K.B., Deshmukh, A.A., Hiwalkar, S.M., Kumbhar, G.M., & Ladke, V.S. (2025). Lauric acid's potential as an apoptotic agent in oral cancer: unravelling the mechanism through an in vitro analysis. Biomedical and Biotechnology Research Journal, 9(3), 309–314. https://doi.org/10.4103/BBRJ.BBRJ_237_25

Chandra, K., Nautiyal, B.P., & Nautiyal, M.C. (2013). Ethno-botanical resources as supplementary foods and less known wild edible fruits in District Rudraprayag, Uttarakhand, India. Journal of Human Ecology, 42(3), 259–271. https://doi.org/10.1080/09709274.2013.11906600

Changade, J.V., Thanvi, H., Raut, C., Chavan, M., Prasad, P., & Ladke, V.S. (2024). In vitro evaluation of anti-cancer potential of different solvent extracts derived from *Clerodendrum*

infortunatum Linn against cervical cancer. Asian Pacific Journal of Cancer Prevention, 25(3), 1065–1075. https://doi.org/10.31557/APJCP.2024.25.3.1065

Chrysargyris, A. (2024). It runs in the family: the importance of the Lamiaceae Family Species. Agronomy, 14(6), 1274. https://doi.org/10.3390/AGRONOMY14061274

Dahiya, S., Batish, D.R., & Singh, H.P. (2020). Ethnobotanical, phytochemical and pharmacological aspects of Bengal Pogostemon (*Pogostemon benghalensis*). Journal of Herbmed Pharmacology, 9(4), 318–327. https://doi.org/10.34172/JHP.2020.40

Kanase, M., Dharkar, N., & Ladke, V.S. (2025). Evaluating the efficacy of Benincasa Hispida seed extract in inhibiting the proliferation of a human triple negative breast cancer cell line. Asian Pacific Journal of Cancer Prevention, 26(2), 471. https://doi.org/10.31557/APJCP.2025.26.2.471

Kumbhar, G.M., Jadhav, A.D., Kheur, S., & Ladke, V.S. (2024). Andrographolide demonstrates anti-proliferative activity in oral cancer by promoting apoptosis, the programmed cell death process. Iranian Journal of Basic Medical Sciences, 27(10), 1300–1308. https://doi.org/10.22038/IJBMS.2024.76691.16599

Kumbhar, G.M., Suryawanshi, P., & Ladke, V.S. (2025). Wortmannin exhibits anticancer activity in oral cancer cell line by targeting PI3K, AKT and mTOR pathway. Avicenna Journal of Phytomedicine. 16(2), 78–98. https://doi.org/10.22038/AJP.2025.26226

Ladke, V.S., Kumbhar, G.M., Joshi, K., & Kheur, S. (2022). Systemic explanation of Glycyrrhiza glabra's analyzed compounds and anti-cancer mechanism based on network pharmacology in oral cancer. Journal of Oral Biosciences, 64(4), 452–460. https://doi.org/10.1016/j.job.2022.09.002

Momin, S., Jadhav, M., & Gurav, R. (2026). Exploring phytochemistry, antioxidant potential, essential oil profiling and bioactive profiling of *Pogostemon mollis* Benth. through GC–MS and UPLC-QTOF-MS/MS. Scientific Reports, 16(1), 6277. https://doi.org/10.1038/s41598-026-35989-2

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65(1–2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Newman, D.J., & Cragg, G.M. (2016). Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products, 79(3), 629–661. https://doi.org/10.1021/ACS.JNATPROD.5B01055

Nieto, G. (2017). Biological activities of three essential oils of the Lamiaceae Family. Medicines, 4(3), 63. https://doi.org/10.3390/MEDICINES4030063

Panda, S.K., Van Puyvelde, L., Mukazayire, M.J., & Gazim, Z.C. (2022). Editorial: Ethnopharmacology of the Lamiaceae: Opportunities and challenges for developing new medicines. Frontiers in Pharmacology, 13, 961486. https://doi.org/10.3389/FPHAR.2022.961486/TEXT

Patel, M., Antala, B., Dowerah, E., Senthilkumar, R., & Lahkar, M. (2014). Antitumor activity of *Pogostemon benghalensis* Linn. on Ehrlich ascites carcinoma tumor bearing mice. Journal of Cancer Research and Therapeutics, 10(4), 1071–1075. https://doi.org/10.4103/0973-1482.138014

Sathishkumar, K., Chaturvedi, M., Das, P., Stephen, S., & Mathur, P. (2022). Cancer incidence estimates for 2022 & projection for 2025: Result from National Cancer Registry Programme, India. Indian Journal of Medical Research, 156(4), 598–607. https://doi.org/10.4103/IJMR.IJMR_1821_22

Bhandari, S.C. (1993). *Vanaushadhi Chandrodaya*. Varanasi: Chaukhamba Sanskrit Sansthan.

Singh, S., & Agrawal, N. (2024). Exploring the pharmacological potential and bioactive components of Pogostemon cablin (Blanco) Benth, traditional Chinese medicine. Pharmacological Research - Modern Chinese Medicine, 10, 100382. https://doi.org/10.1016/J.PRMCM.2024.100382

Singh, S., Sharma, B., Kanwar, S.S., & Kumar, A. (2016). Lead phytochemicals for anticancer drug development. Frontiers in Plant Science, 7, 1667. https://doi.org/10.3389/FPLS.2016.01667

Sinnarkar, S., Suryawanshi, P., Dilip, A., Bhawalkar, J., & Ladke, V. (2024). Galangin promotes apoptosis by upregulating the pro-apoptotic gene BAX in triple-negative breast cancer. Journal of the Egyptian National Cancer Institute, 36(1), 41. https://doi.org/10.1186/S43046-024-00246-Y

Sporn, M.B., & Liby, K.T. (2005). Cancer chemoprevention: scientific promise, clinical uncertainty. Nature Clinical Practice. Oncology, 2(10), 518–525. https://doi.org/10.1038/NCPONC0319

Desai, V. G. (1975). Aushadhi Sangrah (1st ed.).

Wyreogonekbska, A., Gach, K., Lewandowska, U., Szewczyk, K., Hrabec, E., Modranka, J., et al. (2013). Anticancer activity of new synthetic α-methylene-δ-lactones on two breast cancer cell lines. Basic & Clinical Pharmacology & Toxicology, 113(6), 391–400. https://doi.org/10.1111/BCPT.12120