Indian Journal of Natural Products and Resources
Vol. 16(1), March 2025, pp 80-88
DOI: 10.56042/ijnpr.v16i1.13721

In vitro anticancer effects of ethanolic extract of *Pandanus odorifer* flower on HT-29 colon cancer cells by targeting p53 pathway

Meenakshi Kaniyur Chandrasekaran[1], Manikandan Vani Raju[1],
Rathi Muthaiyan Ahalliya[1,2]* and Gopalakrishnan Velliyur Kanniappan[3]

[1]Department of Biochemistry, [2]Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore- 641021, Tamil Nadu, India

[3]Department of Physiology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS) Chennai 602105, Tamil Nadu, India

Received 13 September 2024; revised received 30 January 2025; accepted 21 February 2025

Colon cancer is the third most commonly diagnosed malignancy and the second leading cause of fatalities worldwide. Plant-derived constituents decrease colon cancer by reducing tumours, chemotherapeutic effects and exerting their actions at the molecular level. The objective of the study was to investigate the cytotoxic potential of ethanolic extract of *Pandanus odorifer* flower (EEPO) on HT-29 colon cancer cells. The cytotoxic assay, enzymatic antioxidant determination, gene expression and cell cycle analysis were performed to determine the effect of EEPO on HT-29 cells. The cytotoxic activity showed an IC_{50} value of 12.2±0.19 µg/mL for 5- Fluorouracil (5-FU) and 17.6±0.21µg/mL for EEPO on HT-29 cells, which indicates potent cytotoxic potential. The determination of enzymatic antioxidants revealed a decrease in superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels, along with an increase in lipid peroxidation (LPO), leading to apoptosis in EEPO treated cells. Further, the cell cycle analysis exhibited interphase arrest at the G1/S phase, which prevented the cells from proliferation. The RT-PCR showed the upregulation of p53-Upregulated Modulator of Apoptosis (PUMA) gene and downregulation of Bcl-2 gene, thereby enhancing apoptosis through the p53 pathway. These outcomes suggest that EEPO was an effective natural agent for triggering apoptosis on HT-29 colon cancer cells. By targeting the p53 pathway, EEPO shows a potential as a natural compound therapeutic agent. The findings highlight the wider significance of EEPO in creating potent plant-based treatments for colon cancer.

Keywords: Antioxidant, Apoptosis, Colon cancer, Cytotoxicity, *Pandanus odorifer* flower

IPC code; Int. cl. (2021.01)– A61K 36/00, A61K 133/00, A61P 35/00

Introduction

Cancer is a condition characterized by abnormal signalling and metabolism, which leads to uncontrolled cell division and survival. A wide range of substances, factors and circumstances have been identified as underlying causes for onset and progression of this condition[1]. Colon cancer is the third most frequent malignancy and the second leading cause of cancer death globally. More than 1.9 million colon cancer cases were reported in 2020, with almost 0.9 million individuals dying as a result of colon cancer worldwide[2]. By 2040, colon cancer is expected to cause 3.2 million new cases and 1.6 million deaths, with the majority of cases occurring in countries with a high Human Development Index[3]. Herbal medicines are gaining popularity as a possible source of anticancer agents due to their availability, affordability, low cost, few or no side effects, broad utility, and effectiveness in therapy, which has encouraged scientific research. For these reasons, the World Health Organisation encourage the use of traditional medicines that are effective and non-toxic[4]. The search for natural treatments derived from plant sources with antioxidant qualities has grown into a substantial area of research[5]. *Pandanus odorifer* (Forssk.) Kuntze, also known as *Pandanus odoratissimus* L.f., is a small tree from the Pandanaceae family. It has been used in Sri Lanka, Taiwan, and India to cure a variety of conditions through traditional medicine such as Siddha and Ayurveda. The flower of this plant was used to treat diabetes, skin problems, asthma, urinary tract ailments, and syphilis[6]. While these traditional treatments demonstrate the pharmacological potential,

*Correspondent author
Email: rathiajith@gmail.com

its anticancer activities remain unexplored. *P. odorifer* was selected for this study due to its rich phytochemical profile which includes bioactive components such as flavonoids and phenols terpenoids prominent for their antioxidant and anticancer activities[7].

The enzymatic antioxidants are mostly composed of SOD, GPx and catalase. When exposed to oxidative stress, such antioxidant enzymes are stimulated or triggered to protect against oxidation. SODs convert oxygen into molecular oxygen and Hydrogen peroxide, regulating reactive oxygen species (ROS) levels and reducing toxicity. After the dismutation of oxygen by SOD to form hydrogen peroxide, it will decompose into oxygen and water by catalase and GPx. Similarly, LPO also plays an important role in the progression of cancer. LPO is a multifaceted process wherein oxidants target carbon-carbon double bonds found in lipids, resulting in the oxidative breakdown of lipids, the production of lipid peroxides, and ultimately, a change in cellular conditions. It is a crucial component of lipid metabolism and is necessary for both human health and the proper operation of cells. Lipid peroxidation in the context of cancer promotes genomic instability, activates pro-inflammatory pathways, and induces immune evasion, all of which aid in the tumour's growth[8].

According to the Fearon and Vogelstein model, p53 is the gene which is involved in the malignant stage of colon cancer[9]. p53 is a tumour suppressor gene that can cause apoptosis by directly perforating mitochondria in the cytoplasm or by triggering gene expression in the nucleus. It has been demonstrated that PUMA, a downstream target of p53 and Bcl-2 family member, is critical in apoptosis caused by both nuclear and cytoplasmic p53[10]. During DNA damage, the tumour suppressor and transcription factor p53 attaches to specific DNA sequences in the promoter regions of its target genes. p53 activates these genes, resulting in cell cycle arrest, apoptosis, DNA repair, and other responses. According to estimates, the human genome contains at least several hundred p53-binding sites. The PUMA promoter region has two p53-binding sites that can be directly bound and transactivated by p53. Nuclear p53 stimulates the production of PUMA, which binds to anti-apoptotic proteins Bcl-2 and Bcl-XL via its BH3 domain and removes their inhibition on the proapoptotic Bcl-2 family (Bax). This results in the activation of caspases which finally leads to apoptosis[11]. This study aims to investigate the cytotoxic activity of ethanolic extract of *P. odorifer* flower on HT-29 colon cancer cells lines and to analyze its effect on enzymatic antioxidants. Additionally, this research explores the effect of EEPO on cell cycle arrest and gene expression with a specific focus on the p53 pathway. To the best of our knowledge, this study represents the first investigation into the molecular mechanisms of EEPO on HT-29 colon cancer cells.

Materials and Methods

Collection of the sample

P. odorifer flower was collected from Pollachi, Coimbatore during the monsoon season and was authenticated by Dr. M. U. Sharief, (BSI/SRC/5/23/2023/Tech-800), Botanical Survey of India, TNAU campus, Coimbatore.

Preparation of sample extract

P. odorifer flowers were rinsed thoroughly and shade-dried. Subsequently, the dried sample was ground into powder and kept in an airtight container for future use. The sample was extracted with organic solvent using soxhlet method. The powdered sample was packed into the soxhlet and extracted using ethanol in the ratio of 1:5. The extraction process was carried out at a controlled temperature of 60°C for 6 hours to ensure optimal extraction of bioactive compounds. The excess solvent was allowed to evaporate and the dried extract was stored for further studies.

Cell culture and treatment

The HT-29 cells were procured from National Centre for Cell Science (NCCS), Pune, India. The cell lines were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM) with 10% inactivated Fetal Bovine Serum (FBS), 100 IU/mL of penicillin and 100 µg/mL of streptomycin in a moist condition with 5% CO_2 until 85% confluent. The cells were separated using a trypsin solution containing trypsin (0.2%), Ethylenediamine tetraacetic acid (EDTA) (0.02%), and glucose in Phosphate-buffered saline (PBS) (0.05%)[12].

Cytotoxic activity

The MTT assay was used to detect the cytotoxic activity of EEPO in HT-29 cells[13]. The cells (1×10^4 / well) were plated in 96-well plates with 0.2 mL of media per well. Incubate the plate for 72 hours in an incubator with 5% CO_2. The samples were then mixed

at different concentrations (3.16, 7.80, 15.60, 31.20, 62.50, 125 µg/mL) in 0.1% dimethyl sulfoxide (DMSO) for 48 hours in a 5% CO_2 incubator. Use an inverted microscope at 40X magnification to view the images. After removing the sample solution, 20 µL of 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) reagent was applied to each well. The absorbance at 540 nm was used to determine the number of viable cells. 5-FU at equivalent concentrations was used as the positive control for cytotoxicity. The percentage of cell viability of HT-29 cells treated with the EEPO was assessed and calculated using the formula

$$\% \text{ cell viability} = \frac{A_{540} \text{ of extract treated cells}}{A_{540} \text{ of control cells}} X\,100\%$$

Determination of enzymatic assays

Superoxide dismutase

The superoxide dismutase enzyme activity on the HT-29 cells was determined by the method followed by[14]. 0.1 mL of the sample with concentrations (15,30 and 60 µg/mL) was mixed with 0.5 mL of distilled water. To this, 0.25 mL of ethanol and 0.15 mL of chloroform were added. The mixture was stirred for one minute and centrifuged at 2000 rpm. The cells were seeded in six-well plate. The enzyme in the supernatant was determined. The reaction was initialized by adding 0.4 mL epinephrine, and the change in optical density per minute at 470 nm was used to calculate superoxide dismutase activity.

$$\% \text{ of superoxidedismutase} \frac{\text{Absorbanceof blank - Absorbanceof test}}{\text{Absorbanceof blank}} X\,100$$

Lipid peroxidation

The cells were seeded in a six-well plate with 2 mL medium and incubated for 24 hours at 37°C. The samples (15, 30 and 60 µg/mL) were introduced to a fresh medium and incubated for 48 hours. The cells were centrifuged at 10,000 rpm for 10 minutes at 4°C and rinsed with PBS. After that, the lysed cells were treated with thiobarbituric acid (TBA) to produce the Malondialdehyde thiobarbituric acid (MDA TBA) mixture. The MDA TBA mixture has been determined spectrophotometrically at 532 nm[15].

Glutathione peroxidase

The glutathione peroxidase enzyme assay was done using the method followed by[16] with minor alterations. The cells were seeded in six-well plates. A sample with concentrations (15, 30 and 60 µg/mL) was mixed with 0.2 mL of EDTA and 0.1 mL of sodium azide. Add 0.1 mL of hydrogen peroxide (H_2O_2) and 0.2 mL of reduced glutathione to 0.4 mL of PBS. The mixture was incubated at 37°C for 10 minutes and 0.5 mL of trichloroacetic acid (TCA) and 3 mL of disodium hydrogen phosphate (Na_2HPO_4) were added. Finally, 1 mL of 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) solution was added. The optical density was measured at 420 nm. The glutathione peroxidase activity was reported as min/mg protein.

Cell cycle analysis

The HT-29 cells were grown in T25 flasks. When the cells had attained confluence, they were transferred to six-well plates. After cell adhesion (48-72 hours), cells were treated with various doses of the sample for 24 hours. The cells were collected by trypsinization with Trypsin Phosphate Versene Glucose (TPVG) solution. The cells were centrifuged at 1200 rpm for 5 minutes at room temperature and the supernatant was discarded. The cells were then rinsed with PBS. The cell pellet was reintroduced in 300 µL of PBS. A falcon tube was filled with 700 µL of ice-cold 100% ethanol, then 300 µL of the sample was added drop by drop. The cells were fixed in ethanol and kept overnight at 4°C. During flow cytometry analysis, ethanol-fixed cells were washed once or twice with PBS. Then, 556 µL (0.5%) of Triton X and 20 µL (0.1 mg/mL) of Rnase were added and incubated for one hour. After one hour, 24 µL (40 µg/mL) of propidium iodide was added and incubated in the dark. After incubation, the sample was examined with a flow cytometer instrument (Facs JAZZ, California)[17].

Reverse transcription polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated from EEPO treated HT-29 cells using TRIzol reagent, following manufacturer's instructions. The RNA concentration was measured using the spectrophotometric technique. Then, 1 µg of total RNA was used for reverse transcription reactions. The cDNA has been precipitated and amplified with primer sequences (Table 1) to enable effective and targeted amplification. Primer sets targeting PUMA (pro-apoptotic) and Bcl-2 (anti-apoptotic) and GAPDH (housekeeping gene) were developed for cDNA amplification[17].

Statistical analysis

Statistical analysis was carried out using GRAPHPAD PRISM 9. The results of the

Table 1 — Primers for PUMA, Bcl-2 and GAPDH genes with accession number

Gene	Gene Accession Number	Forward primer	Reverse primer
PUMA	NM_001127240.3	GGGCAGGAAGTAACAATGAGA	CTCCCTGGGGCCACAAATC
Bcl-2	NM_000633.3	AAAAATACAACATCACAGAGGAAGT	GTTTCCCCCTTGGCATGAGA
GAPDH	NM_002046.7	TTTTGCGTCGCCAGCC	ATGGAATTTGCCATGGGTGGA

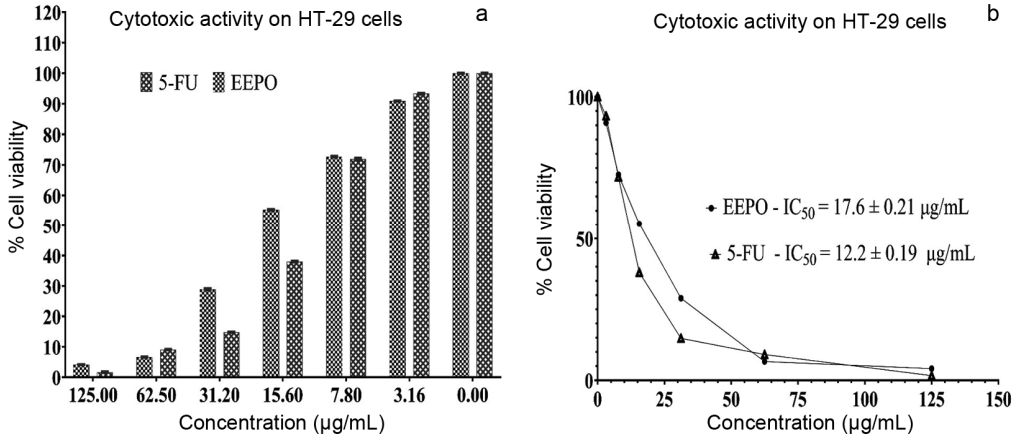

Fig. 1 — a) Graphical representation of cytotoxic activity on HT-29 cells treated with various concentrations of EEPO and 5-FU, as assessed by the MTT assay. The values show the percentage of viable cells compared to untreated controls. Error bars represent the mean ± SD from three different studies (n=3), and b) The determination of IC_{50} values for EEPO and 5-FU. The IC_{50} value indicates the concentration needed to inhibit cell viability by 50%. EEPO and 5-FU, respectively.

experiments were represented by the mean and standard deviation (SD) of triplicates. One-way ANOVA followed by Dunnett's test was used to evaluate variance and identify significant differences between means with a significance threshold set at p <0.05. The probability levels were indicated as p =<0.0001 (****). For each assay, the specific statistical tests and parameters used have been integrated into the results.

Results and Discussion

Percentage of yield

P. odorifer flowers were extracted with ethanol yielding a 13% extract based on the initial mass of the flower. This yield percentage represents the amount of extract obtained in relation to the starting material and demonstrates the efficiency of the extraction process. The discovery of plant-derived anticancer medicines has created widespread interest in investigating the efficacy of many different natural products. The plant and fungal extracts or bioactive substances have a variety of anti-tumor actions. Natural substances may not be utilized as medications, but they prompted researchers to design and develop new anticancer agents[18]. Recent study highlights the necessity of improving extraction procedures to improve the yield and bioavailability of active compounds, particularly those with anti-cancer potential[19].

Cytotoxic activity

The cytotoxic effect of 5-FU and EEPO on HT-29 cells were assessed and are shown in Fig. 1a. Both 5-FU and EEPO exhibited strong cytotoxic effects on HT-29 cells in a dose-dependent way. Fig. 1b relates the IC_{50} values for 5-FU and EEPO. The amount needed to reduce cell viability by 50% was indicated by the IC_{50} values for 5-FU and EEPO, which were 12.2±0.19 and 17.6±0.21 µg/mL, respectively. The inverted microscopic images of cytotoxic activity were given in Fig. 2. The viability and scalability of generating the extract for additional study and possible medicinal uses depend heavily on the process of extraction efficiency[20]. The bioactive compounds may be present in considerable amounts based on the effectiveness of EEPO in biological studies such the MTT assay, enzymatic assays, RT-PCR analysis and cell cycle analysis. The MTT assay works by reducing MTT intracellularly to purple formazan granules. Within the cells, the reduction of MTT can be achieved by oxidoreductase and dehydrogenase enzymes and electron donors (primarily NADPH) at various levels of the glycolytic routes to the mitochondrial electron transport mechanism[21]. The

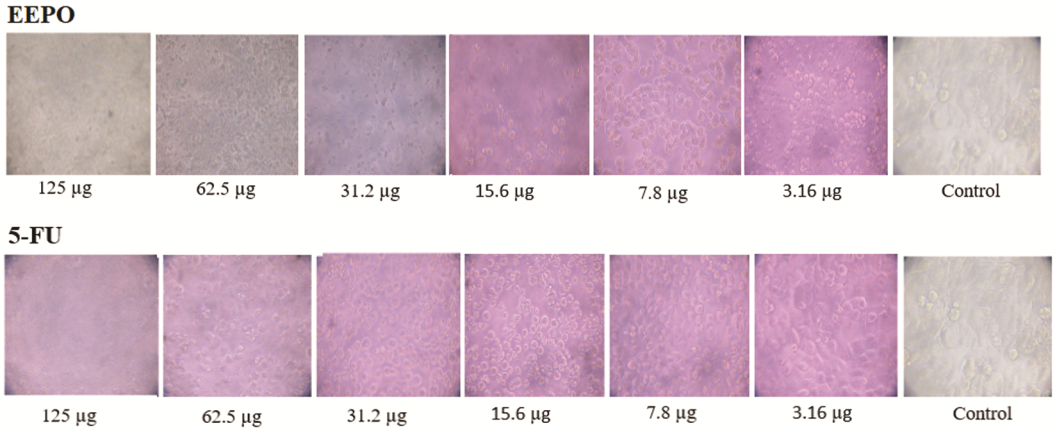

Fig. 2 — Microscopic images of HT-29 cells treated with EEPO and 5-FU.

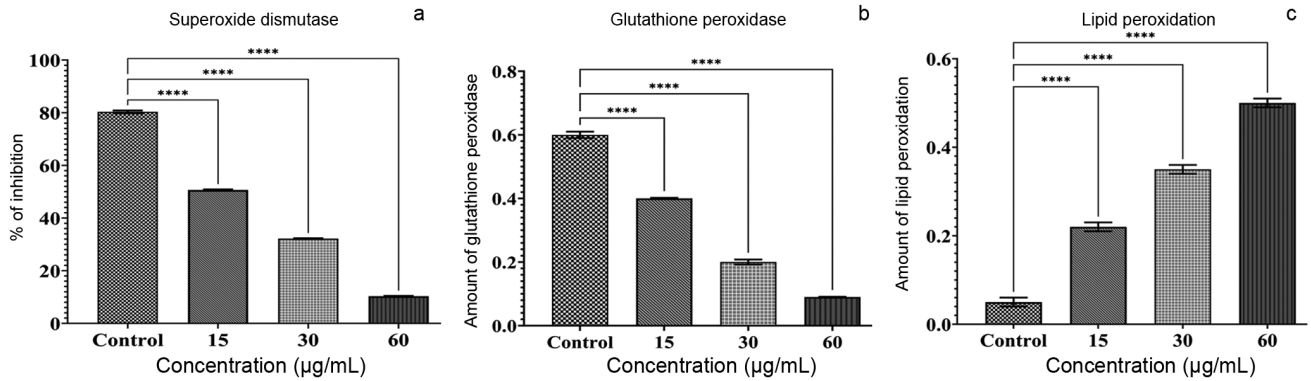

Fig. 3 — a) SOD activity in HT-29 cells treated with increasing doses of EEPO. The data show a dose-dependent decrease in SOD activity, indicating an impaired antioxidant defense mechanism. b) Glutathione peroxidase activity following EEPO administration. GPx levels fall considerably with greater EEPO concentrations, indicating increased oxidative stress, and c) Lipid peroxidation levels in HT-29 cells treated with EEPO. Elevated LPO levels imply increased oxidative damage to cell membranes, which contributes to apoptosis. Values are shown as mean ± SD (n=3).

cytotoxicity reported with EEPO could be attributable to a number of processes, including the activation of apoptosis, inhibition of cell proliferation, and alteration of cell metabolism. This study shows that EEPO has substantial cytotoxic action against HT-29 colon cancer cells, with an IC_{50} value similar to 5-FU. 5-FU is a popular chemotherapeutic drug observed for its efficacy against a variety of cancer cell lines, including colon cancer. The investigation found that 5-FU had a lower IC_{50} value (12.2±0.19 µg/mL) than EEPO (17.6±0.21 µg/mL), which is consistent with its proven potency. However, the similar IC_{50} values show that EEPO has strong cytotoxic potential and requires more exploration. These findings are consistent with prior research showing the efficiency of natural plant extracts in targeting cancer cells[22].

Determination of enzymatic assays

Fig. 3a represents the effects of varying EEPO concentrations on superoxide dismutase activity. The levels of SOD were decreased when the concentration of EEPO increased. The control group had the highest percentage of inhibition, with a gradual decrease at the dosages of 15, 30, and 60 µg/mL. Likewise, the control group had the highest GP_X level, around 0.6 min/mg protein. The treatment with EEPO of 15 µg/mL drastically lowered GP_X levels to around 0.4 min/mg protein. Similarly, the treatments at 30 µg/mL and 60 µg/mL resulted in levels of 0.20 and 0.09 min/mg protein, respectively as shown in Fig. 3b. LPO levels increased with rising treatment concentrations as given in Fig. 3c. The control group exhibited the lowest level, around 0.05. EEPO treated with 15 µg/mL significantly increased LPO levels to about 0.22. The levels increased further after treatments at 30 µg/mL and 60 µg/mL reaching 0.35 and 0.50 respectively. This demonstrates that high lipid oxidation alters the physical characteristics of cellular membranes and proteins, leading to the initiation of apoptosis. The cytotoxic effect of EEPO

against HT-29 cells is highly associated with the regulation of enzymatic antioxidant activities, indicating that oxidative stress is an important factor in its mechanism of action[23]. An increase that was observed in LPO levels suggests that the EEPO treatment causes considerable oxidative stress in HT-29 cells. LPO is a process in which free radicals, namely ROS, damage polyunsaturated fatty acids in cell membranes, causing cell damage and elevated MDA levels[24]. The higher MDA levels found in this study reflect the severity of EEPO induced oxidative damage. The increase in LPO indicates that the equilibrium between ROS production and antioxidant defence is broken, resulting in oxidative stress. This oxidative stress may harm cellular components such as DNA, lipids and proteins eventually leading to cell death[25]. The lower activity of GPx and SOD in EEPO-treated cells suggests a decreased antioxidant defence mechanism. GPx and SOD are important enzymes that prevent cells from oxidative damage by neutralizing ROS. Reduced GPx activity in EEPO-treated cells indicates an inability to properly eliminate lipid peroxide and hydrogen peroxides, resulting in ROS buildup and increased oxidative stress. Similarly, the decrease in SOD activity following EEPO treatment implies a lower ability to remove superoxide radicals, which contributes to oxidative damage and LPO. Many plant-derived anticancer medicines rely on an oxidative stress-mediated mechanism[26].

Cell cycle analysis

The flow cytometry was used to analyze the cell cycle arrest of HT-29 cells treated with control and EEPO was shown in Fig. 4a and b. The occurrence of cells in distinct phases was determined and expressed as live cells (P1), dead cells (P2), interphase (P3) and mitotic phase (P4). The findings show that the proportion of living cells (P1) in the group treated

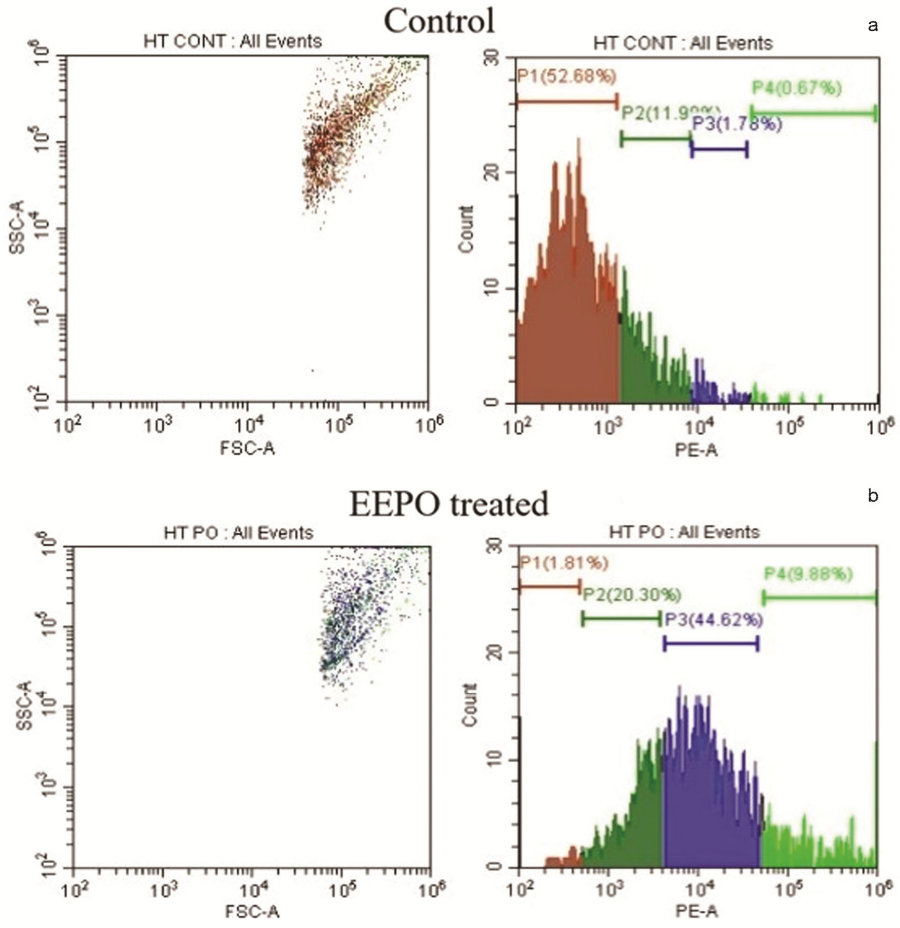

Fig. 4 — a) Flow cytometric analysis of HT-29 cells under control conditions, showing normal cell cycle distribution. Peaks correspond to live cells (P1), dead cells (P2), interphase (P3) and mitotic phase (P4), and b) Flow cytometric analysis of HT-29 cells treated with EEPO, showing significant alterations in cell cycle distribution. EEPO treatment induces G1/S phase arrest (P3) and reduces the proportion of live cells (P1).

with the EEPO is significantly less than in the control. In contrast, the treated group shows a higher percentage of arrest in the P3 phase. The apparent rise in P3 could indicate that the EEPO causes a G1/S phase arrest, stopping cells from advancing to the next stage of the cell cycle. The flow cytometry study revealed substantial information about the impact of EEPO on the cell cycle of HT-29 cells. This study showed cell cycle arrest at interphase which is early-stage arrest. The research on HT-29 colon cancer cells by[27] showed cell cycle arrest at G2/M phase. Thus, controlling cell cycle progression by activating cell cycle arrest may be a viable technique for cancer treatment. When DNA damages, the cell cycle is arrested by activating cellular checkpoints such as G1/S or G2/M phase until DNA errors are rectified. Yet, if DNA damage is severe and irreversible, apoptosis occurs instead of cell cycle halt. As biological defensive mechanisms, cell cycle arrest and apoptosis work in combination. Indeed, tumour cells are viewed as a collection of altered cells that persist in the presence of cell death signals. As a result, inducing cell cycle arrest or apoptosis is seen to be a promising chemopreventive technique[28]. This study found that EEPO alters the expression of vital apoptotic regulators, hence increasing apoptosis in HT-29 cells. This is similar to findings from previous research indicating that plant-derived chemicals can modify cell cycle checkpoints, inhibiting tumour growth[29].

RT-PCR analysis

The expression levels of the PUMA and Bcl-2 genes were assessed by RT-PCR analysis at various treatment concentrations (15, 30, and 60 µg/mL) in comparison to the control. The RT-PCR results for PUMA, Bcl-2, and GAPDH at various treatment doses are shown in Fig. 5b. The intensity of the bands increases as the treatment concentration increases, showing that the expression of PUMA is upregulated. The intensity of the bands reduces as the treatment concentration increases, indicating that the expression of Bcl-2 is being down regulated Fig. 5a indicates the expression levels of PUMA and Bcl-2 in relation to GAPDH. The expression level of PUMA increases particularly with greater treatment concentrations, while the expression level of Bcl-2 decreases significantly. Fig. 5c shows the fold change levels in which PUMA exhibits a substantial rise with higher treatment doses, indicating increased expression and Bcl-2 shows a significant drop with increasing treatment concentrations, indicating lower expression. The treatment promotes dose-dependent overexpression of the pro-apoptotic gene PUMA and downregulation of the anti-apoptotic gene Bcl-2, which enhances apoptosis via the p53 pathway. The RT-PCR study revealed a dose-dependent rise in PUMA expression and a consistent decrease in Bcl-2 levels, indicating a strong pro-apoptotic effect. These findings are consistent with previous research on p53-mediated apoptotic pathways, as described for other plant-derived substances such as berberine and quercetin. Such comparisons highlight the therapeutic potential of EEPO as a natural drug that targets apoptotic pathways in colon cancer cells[30,31].

PUMA was first identified as a p53 transcriptional regulator and an effective apoptosis stimulant in a variety of cancer cells. PUMA is found in the

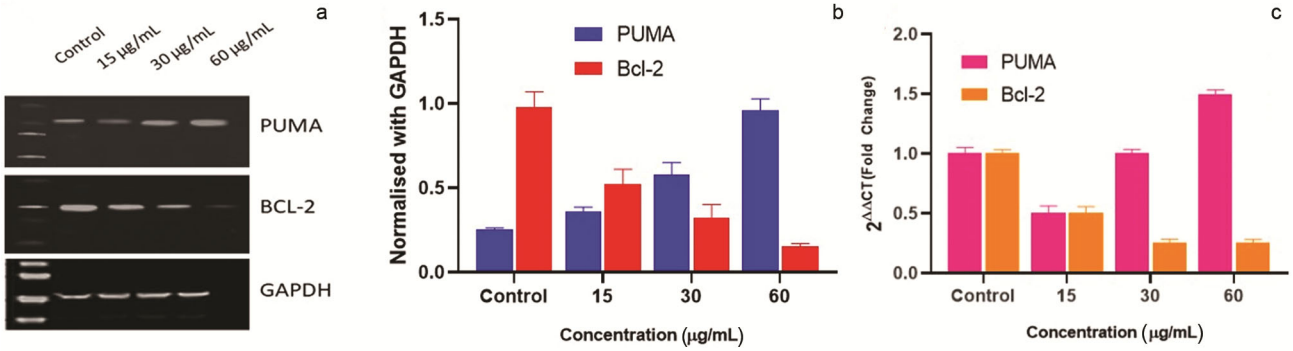

Fig. 5 — a) Agarose gel electrophoresis for PUMA, Bcl-2 and GAPDH genes. Bands represent gene expression levels across varying EEPO concentrations, b) Graphical representation of PUMA and Bcl-2 gene expression relative to GAPDH. PUMA expression increases with higher EEPO concentrations, while Bcl-2 expression decreases, indicating activation of pro-apoptotic pathways, and c) Fold change analysis of PUMA and Bcl-2 expression levels. Data show a significant dose-dependent increase in PUMA expression and decrease in Bcl-2 expression, emphasizing the apoptotic mechanism induced by EEPO. Error bars indicate mean ± SD from three independent experiments. Values are shown as mean ± SD (n=3).

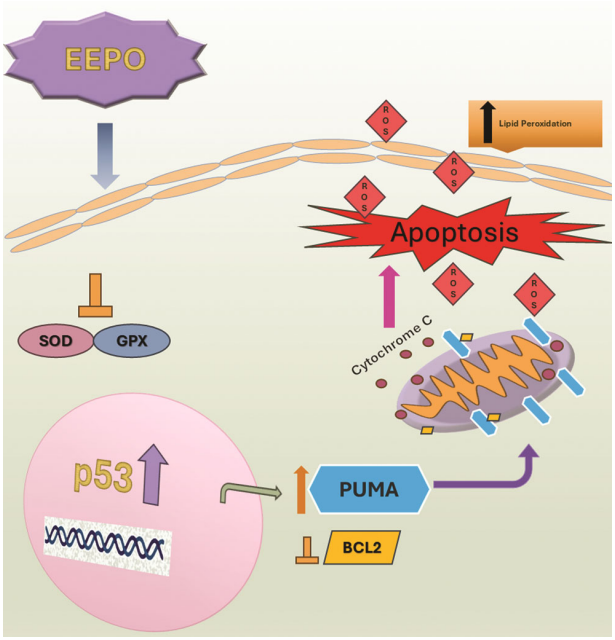

Fig. 6 — Schematic representation of the apoptotic mechanism induced by EEPO in HT-29 cells. EEPO upregulates pro-apoptotic PUMA and downregulates anti-apoptotic Bcl-2 via the p53 pathway, leading to mitochondrial dysfunction, activation of caspases, and cell death. This mechanism is supported by RT-PCR and enzymatic assay findings.

mitochondria and promotes apoptosis via the family of Bcl-2 proteins and a mitochondrial pathway[32]. The study by[33] showed the upregulation of PUMA gene and downregulation of Bcl-2 gene by inducing apoptosis in treated breast cancer cells. Hence, the phytocompounds present in the EEPO induces apoptotic mechanism as shown in Fig. 6. The observed apoptotic effects of EEPO are likely mediated through the upregulation of PUMA and downregulation of Bcl-2, as supported by RT-PCR analysis. Further *in vivo* and molecular studies are necessary to confirm these mechanisms. This finding is consistent with prior studies on natural chemicals that modulate apoptosis via p53-dependent pathways[34,35].

Conclusion

This study emphasizes the substantial anticancer activity of EEPO against HT-29 colon cancer cells. This study provides an excellent basis for the development of plant-based anticancer therapies and emphasizes the importance of natural products in drug discovery and development. The findings show that EEPO has significant cytotoxic effects, with an IC_{50} value similar to the proven chemotherapeutic drug 5-FU, indicating that it has prospective anticancer properties. The apparent oxidative stress, which is expressed by higher LPO levels along with reduced antioxidant enzyme activity GPx and SOD demonstrates that EEPO promotes cytotoxicity. The enhanced oxidative stress and impairment of cellular antioxidant defenses are important in understanding the extract's anticancer properties. In addition, effects of EEPO on the cell cycle and apoptotic pathways point to its efficacy as a therapeutic agent. To ensure the broader relevance of these results, more research is necessary to understand the precise molecular mechanisms of action and identify the compounds that are responsible for the identified benefits. Overall, this work emphasizes the importance of plant-derived compounds in generating innovative anticancer therapeutics and promotes more research into natural products in the search for effective cancer treatments. Furthermore, the study highlights the importance of conducting preclinical and clinical trials to evaluate its safety, efficacy, and translational prospects in drug development.

Conflict of interest

There is no conflict of interest regarding the publication of this manuscript.

References

1 Upadhyay A, Cancer: An unknown territory; rethinking before going ahead, *Genes Dis*, 2020, **8**(5), 655–661, doi: 10.1016/j.gendis.2020.09.002.

2 Roshandel G, Ghasemi-Kebria F and Malekzadeh R, Colorectal cancer: Epidemiology, risk factors, and prevention, *Cancers*, 2024, **16**(8), 1530, doi: 10.3390/cancers16081530.

3 Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag C J, *et al.*, Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN, *Gut*, 2022, **72**(2), 338–344, doi: 10.1136/gutjnl-2022-327736.

4 Jain S, Dwivedi J, Jain P K, Satpathy S and Patra A, Medicinal plants for treatment of cancer: A brief review, *Pharmacogn J*, 2016, **8**(2), 87–102, doi: 10.5530/pj.2016.2.1.

5 Dhanapal D P, Chandrasekaran M K, Raju M V, Ahalliya R M, Sundarraj R, *et al.*, Hepatoprotective effects of ethanolic extract of *Corallocarpus epigaeus* roots on nitrobenzene-induced liver injury in Wistar rats, *J Herbs Spices Med P*, 2024, **31**(1), 1–17, doi: 10.1080/10496475.2024.2423279.

6 Sathasivampillai S V, Shanmugalingam V and Sebastian P R, Pharmacological activities of extracts and isolated phytochemical constituents of *Pandanus odorifer* (Forssk.) Kuntze, *Gümüşhane univ sağlık bilim derg*, 2021, **10**(3), 574–580, doi: 10.37989/gumussagbil.948267.

7 Chandrasekaran M, Raju M, Duraisamy B, Kanniappan G and Ahalliya R, Investigating the antioxidant properties and GC-MS profile of Indian native medicinal flower *Pandanus odorifer* and assessing its cytotoxic effects on HT-29 cells, *Int J Ayur Med*, 2024, **15**(3), 650-659, doi: 10.47552/ijam.v15i3.4971.

8 Xiao L, Xian M, Zhang C, Guo Q and Yi Q, Lipid peroxidation of immune cells in cancer, *Front Immunol*, 2024, **14**, doi: 10.3389/fimmu.2023.1322746.

9 Baker S J and Vogelstein B, p53: A tumor suppressor hiding in plain sight, *J Mol Cell Biol*, 2019, **11**(7), 536–538, doi: 10.1093/jmcb/mjz068.

10 Li M, The role of P53 up-regulated modulator of apoptosis (PUMA) in ovarian development, cardiovascular and neurodegenerative diseases, *Apoptosis : Int J Program Cell death*, 2021, **26**(5-6), 235–247, doi: 10.1007/s10495-021-01667-z.

11 Wang P, Yu J and Zhang L, The nuclear function of p53 is required for PUMA-mediated apoptosis induced by DNA damage, *Proc Natl Acad Sci*, 2007, **104**(10), 4054–4059, doi:10.1073/pnas.0700020104.

12 Malsawmdawngliana, Zohmachhuana A, Vabeiryureilai M, Nurpen M T, Lalrinzuali K, *et al.*, Antioxidant efficacy and cytotoxicity of ethanol extract of *Clerodendrum infortunatum* against different cell lines, *Indian J Biochem Biophys*, 2021, **58**, 572–581, doi: 10.56042/ijbb.v58i6.57788.

13 Mosmann T, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, *J Immunol Methods*, 1983, **65**(1-2), 55–63, doi: 10.1016/0022-1759(83)90303-4.

14 Kakkar P, Das B and Viswanathan P N, A modified spectrophotometric assay of superoxide dismutase, *Indian J Biochem Biophys*, 1984, **21**(2), 130–132.

15 Środa-Pomianek K, Michalak K, Świątek P, Poła A, Palko-Łabuz A, *et al.*, Increased lipid peroxidation, apoptosis and selective cytotoxicity in colon cancer cell line LoVo and its doxorubicin-resistant subline LoVo/Dx in the presence of newly synthesized phenothiazine derivatives, *Biomed Pharmacother*, 2018, **106**, 624–636, doi: 10.1016/j.biopha. 2018.06.170.

16 Agahi F, Juan-García A, Font G and Juan C, Study of enzymatic activity in human neuroblastoma cells SH-SY5Y exposed to zearalenone's derivates and beauvericin, *Food Chem Toxicol*, 2021, **152**, 112227, doi: 10.1016/j.fct.2021.112227.

17 Raju M V, Chandrasekaran M K, Rajendran M S, Kanniappan G V, Ahalliya R M, *et al.*, Deciphering the therapeutic, larvicidal, and chemical pollutant degrading properties of leaves-mediated silver nanoparticles obtained from *Alpinia purpurata*, *Bioresour*, 2024, **19**(2), 3328–3352, doi: 10.15376/biores.19.2.3328-3352.

18 Asma S T, Acaroz U, Imre K, Morar A, Shah S R A, *et al.*, Natural Products/Bioactive compounds as a source of anticancer drugs, *Cancers*, 2022, **14**(24), 6203, doi: 10.3390/cancers14246203.

19 Rosário M, Mannochio-Russo H, Santos A, Pinheiro A, Vasconcelos, L, *et al.*, Chemical characterization and evaluation of the anti-cancer potential of flowers from *Fridericia platyphylla* (Bignoniaceae), *J Braz Chem Soc*, 2025, **36**(2), doi: 10.21577/0103-5053.20240132.

20 Ramluckan K, Moodley K G and Bux F, An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the soxhlet extraction method, *Fuel*, 2014, **116**, 103–108, doi: 10.1016/j.fuel.2013.07.118.

21 Ghasemi M, Turnbull T, Sebastian S and Kempson I, The MTT Assay: Utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis, *Int J Mol Sci*, 2021, **22**(23), 12827, doi: 10.3390/ijms222312827.

22 Ilmiyah S, Mamamia A, Permana S, Widodo E, Norahmawati E, *et al.*, Recent advances and mechanism of action of *Anredera cordifolia* (Ten.) Steenis as anticancer approach: A systematic review, *J Pharm Pharmacogn Res*, 2025, **13**(2), 369-380, doi: 10.56499/jppres24.2044_13.2.369.

23 Song W, Hu P, Guo S, Hu J, Song C, *et al.*, Oxidative stress and endoplasmic reticulum stress contribute to *L. paracasei* subsp. paracasei M5L exopolysaccharide-induced apoptosis in HT-29 cells, *Food Sci Nutr*, 2021, **9**(3), 1676–1687, doi: 10.1002/fsn3.2142.

24 Patil K S and Wadekar R R, *Lipid peroxidation: A signaling mechanism in diagnosis of diseases*, (IntechOpen eBooks), 2021, doi: 10.5772/intechopen.99706.

25 Su L J, Zhang J H, Gomez H, Murugan R, Hong X, *et al.*, Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis, *Oxid Med Cell Longev*, 2019, **2019**(1), 5080843, doi: 10.1155/2019/ 5080843.

26 Chávez H, Alvarado A, Asayco-Yataco N, Perez M, García J, *et al.*, Anti-inflammatory effect of the total flavonoid content of the hydroalcoholic extract of the leaves of *Senna alata* (L.) Roxb. in an experimental model of acute inflammation, *J Pharm Pharmacogn*, 2025, **13**(2), 444-458, doi: 10.56499/jppres24.2100_13.2.444.

27 Li X, Qiu Z, Jin Q, Chen G and Guo M, Cell cycle arrest and apoptosis in HT-29 Cells Induced by dichloromethane fraction from *Toddalia asiatica* (L.) Lam, *Front Pharmacol*, 2018, **9**, 629, doi: 10.3389/fphar.2018.00629.

28 Keum Y, Jeong W and Kong A N T, Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms, *Mutat Res Fundam Mol Mech Mutagen*, 2004, **555**(1–2), 191–202, doi: 10.1016/j.mrfmmm.2004.05.024.

29 Doblas G, Catane I, Amoroso V, Ang A, Porquis H, *et al.*, Toxicity, anti-inflammatory, and phytochemical properties of *Christella parasitica* (L.) H.Lev. ex Y.H. Chang in Bukidnon, Philippines, *Palawan Sci*, 2025, **17**(1), 51-60, doi: 10.69721/tps.j.2025.17.1.07.

30 Asgharian P, Tazekand A P, Hosseini K, Forouhandeh H, Ghasemnejad T, *et al.*, Potential mechanisms of quercetin in cancer prevention: Focus on cellular and molecular targets, *Cancer Cell Int*, 2022, **22**, 257, doi: 10.1186/s12935-022-02677-w.

31 Kim J, Lee S K, Park J, Jung M J, An S, *et al.*, Buddlejasaponin IV induces apoptotic cell death by activating the mitochondrial-dependent apoptotic pathway and reducing α2β1 integrin-mediated adhesion in HT-29 human colorectal cancer cells, *Oncol Rep*, 2023, **49**(3).

32 Ming L, Wang P, Bank A, Yu J and Zhang L, PUMA dissociates Bax and Bcl-X(L) to induce apoptosis in colon cancer cells, *J Biol Chem*, 2006, **281**(23), 16034–16042, doi: 10.1074/jbc.M513587200.

33 Sarhan A A M, Boraei A T A, Barakat A and Nafie M S, Discovery of hydrazide-based pyridazino[4,5-b]indole scaffold as a new phosphoinositide 3-kinase (PI3K) inhibitor for breast cancer therapy, *RSC Adv*, 2020, **10**(33), 19534–19541, doi: 10.1039/d0ra02798g.

34 Subhan M, Sanachai K, Sungthong B, Datham S, Ratha J, *et al.*, Comparison *in vitro* and *in silico* studies of phenolic acids and flavonoids on α-glucosidase inhibition, *J Pharm Pharmacogn Res*, 2025, **13**(1), doi: 10.56499/jppres24.2081_13.1.311.

35 Qian S, Wei Z, Yang W, Huang J, Yang Y, *et al.*, The role of BCL-2 family proteins in regulating apoptosis and cancer therapy, *Front Oncol*, 2022, **12**, 985363, doi: 10.3389/fonc.2022.985363.