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Evaluation of Anti-Colorectal Cancer Activity in Methanolic Extract of *Coleus forskohlii* Root

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Abstract: In a multistage process that involves moving from a precancerous lesion to a malignant tumour state, cancer develops when normal cells undergo the change into tumour cells. Colon cancer, also known as colorectal cancer (CRC), is the third most common and second-deadliest malignant tumour in the world. The current study's objective was to assess the anticancer potential of a methanolic extract of *Coleus forskohlii* root on the cancer cell line HT-29 using the MTT assay. A variety of assay methods were used, including morphological investigations, cell growth inhibition, and cell viability. At various concentrations (12.5, 25, 50, 100, and 200 µg/ml), the *Coleus forskohlii* extract was tested against the HT-29 cell line for its ability to inhibit cell proliferation. This study revealed that when concentrations rise the cell growth inhibition levels rise. The conventional medicine Doxorubicin had a growth inhibition of 72.70% at a dose of 5 µg/ml while the lowest growth inhibition was determined to be 9.55% at 12.5 µg/ml and the highest growth inhibition was 71.16% at 200 µg/ml. IC₅₀ was greater than 123.90 µg/ml. According to the study's findings, a human colon adenocarcinoma cell line could be resistant to the anti-colorectal cancer effects of *Coleus forskohlii* root methanolic extract (HT-29).

Keywords: *Coleus forskohlii*, Colorectal cancer, MTT assay, Cell growth inhibition, Cell viability

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Introduction

Approximately one out of every six fatalities globally is due to cancer. Moreover, 70% of deaths in nations with low or middle incomes are due to cancer. New drug discovery options have been made possible by the development of technology and knowledge of neoplastic illness, which in turn

has the potential to reduce the mortality rate from cancer (Zhang *et al.*, 2020). A precancerous lesion transforms into a malignant tumour state as part of the multistage process by which cancer develops. Cancer develops as a result of normal cells turning into tumour cells. These changes are

the result of the interaction between a person's genetic traits and environmental influences, including as physical factors (like ultraviolet radiation), chemical factors (like asbestos and cigarettes), and biological factors (like smoking, other environmental toxins, viruses and bacteria).

The second most lethal kind of cancer and the third most common type of malignant tumour, according to the World Health Organization, is colorectal cancer (CRC), sometimes referred to as colon cancer. 1.8 million new cases of CRC were detected in 2018, and the illness claimed 881,000 lives. This accounted for almost 10% of all newly diagnosed cancer cases and fatalities worldwide (Bray *et al.*, 2018). The chance of survival has risen, and the quality of life for patients has improved because to considerable advancements in cancer therapy. On the other hand, cancer-related fatalities continue to increase (Yadav *et al.*, 2017). Colorectal cancer often presents as an abnormal growth on the inner walls of the colon epithelial cells in its early stages, if it is detected early enough. Surgery may be used to eliminate this tumour. However, if the patient is not treated when the disease is still at an early stage, the cancer cells will spread to other locations on numerous organs after they have already proven resistant to chemotherapy (Gothai *et al.*, 2018).

Cancer may arise when one or more biological mechanisms, such as cell division and apoptosis, that are necessary for the healthy cells' normal growth and proliferation become dysregulated. Apoptosis and cell division are two examples of these processes. Finding the regulatory mechanism(s), specific to cancer cells that control transformation, is the main goal of drug development and candidate screening. After doing this, the next step is to specifically target that mechanism (Wiman and Zhivotovsky, 2017; Matsuoka and Yashiro, 2018; Imran *et al.*, 2017). Currently, the two main alternatives for treating colorectal cancer are chemotherapy with a single medication, such as fluoropyrimidine, or chemotherapy with a combination of drugs, like oxaliplatin, irinotecan, and capecitabine.

Additionally, the optimal therapy for colorectal cancer, which often necessitates surgery, is to completely eradicate the tumour and any metastases. Neoadjuvant or adjuvant chemotherapy or radiation may be required in certain cases before or after surgery to provide the best outcomes in terms of tumour reduction and stabilisation (Xie *et al.*, 2020).

The available colorectal cancer treatments, however, may have harmful side effects and are confusing to understand. As a consequence, scientists are searching for novel treatment approaches with minimal toxicity toward healthy cells and cancer-free cells. The secondary metabolites that may be discovered in medicinal plants are considered as an appealing target for the screening of potential drug candidates in the battle against horrible illnesses like cancer because of their unique structural nature, tremendous variety in their chemical characteristics, and low toxicity (Zaman *et al.*, 2015). The main goal of this work was to use the MTT test to assess the anticancer potential of a methanolic *Coleus forskohlii* root extract on cancer cells obtained from the HT-29 cell line. A variety of assay techniques were used including morphological analyses, cell growth inhibition, and viability testing.

Materials and Methods

Plant components:

The totally developed *Coleus forskohlii* root was harvested in Thanjavur, Tamil Nadu, India in the month of January 2018 using only one herb. A single seed was used to cultivate the plant. The root's identification and verification were established by Dr. S. John Britto, Director of the Rabinat Herbarium and Centre for Molecular Systematics at St. Joseph's College in Trichy, Tamil Nadu, India. It was decided to send one of the voucher specimens to the Rabinat Herbarium at St. Joseph's College in Thiruchirappalli, Tamil Nadu, India.

Preparation of alcoholic extract:

The root of *Coleus forskohlii* was washed many

Table 1: Percentage cell growth inhibition of *Coleus forskohlii* root extract on HT-29 cell line by MTT assay

S. No.	Concentrations (µg/ml)	Absorbance (Optical density)	Cell Viability (%)	Cell growth inhibition (%)
1	12.5	0.734	90.44	9.55
2	25	0.644	79.35	20.64
3	50	0.555	68.45	31.54
4	100	0.400	49.35	50.65
5	200	0.234	28.83	71.16
Standard (5 µg/ml) (Doxorubicin)		0.391	27.29	72.70
Cell Control		0.811	100	0
Half maximum Inhibition Concentration (IC ₅₀)				123.90 µg/ml

times with distilled water to eliminate the pollutants that were discovered on the obtained root. The root was pounded into a powder after being allowed to dry at room temperature. The powder was extracted with methanol for a total of 48 h. The resultant extract was semi-solid once the solvent was entirely withdrawn from the mixture and the pressure was reduced. *Coleus forskohlii* root extract (CFRE), was stored in the freezer until it was required for further analysis.

In vitro evidence of *Coleus forskohlii*'s anti-cancer effectiveness:

To determine if the substance was cytotoxic, the 3-(4,5- dimethylthiazol- 2 -yl) - 2,5 -diphenyltetrazolium (MTT) reduction test was utilised (Aswini *et al.*, 2017). The National Cancer Control Society in Pune provided the HT-29 human colon cancer cell line for purchase. Aliquots of 100 µl of root extracts at various concentrations (12.5, 25, 50, 100, and 200 µg/ml) dissolved in one per cent (v/v) DMSO were added to the appropriate wells that were already containing 100 µl of medium after the samples were incubated for 48 h at 37°C, 5% carbon dioxide, 95% air, and 100% relative humidity. The necessary final sample concentrations were attained as a consequence. A solution containing 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl—tetrazolium bromide (MTT) in phosphate- buffered saline was added to each well after the first 48 h of incubation. The combination was then incubated for 4 h at 37°C.

Following that, 100 µl of DMSO containing 0.1% is poured to each well in order to dissolve the MTT metabolic product. The plate is then shaken at a speed of 150 rpm for 5 min. To find live cells, the sample's absorbance at 570 nm was examined. A graphic depicting the concentration (IC₅₀) required to induce 50% inhibition was created after a series of measurements were made. It was necessary to use a UV Spectrophotometer to get an accurate measurement of the absorbance at 570 nm. For each concentration, three copies of the medium with no materials in it served as the control. At a ten times lower magnification than usual, the inverted microscope (Biolink) was utilised to examine the changes in cellular morphology. The effect that the samples had on the anticancer activity of HT-29 was expressed using the percentage of cytotoxicity. The percentage of cytotoxicity and cell viability was calculated as follow

$$100 - [\text{Abs (sample)} / \text{Abs (control)}] = \% \text{ of cytotoxicity}$$

$$[\text{Abs (sample)} / \text{Abs (control)}] = \% \text{ Cell Viability} \times 100.$$

Results

HT-29 is a human colon cancer cell line that has been widely used in biology and cancer research (Martinez-Maqueda *et al.*, 2015). At different concentrations (12.5, 25, 50, 100, and 200 µg/ml), the *Coleus forskohlii* extract was tested against the HT-29 cell line to see whether it might inhibit cell proliferation. The results of the experiment

demonstrated a relationship between concentration and the degree of cell growth inhibition. The lowest level of growth inhibition was shown to be 9.55% at a dosage of 12.5 µg/ml, while the maximum level was discovered to be 71.16% at a dose of 200 µg/ml. In contrast, the conventional medication doxorubicin showed a growth inhibition level of 72.70% at a dosage of 5 µg/ml. The IC₅₀ value was higher than 123.90 µg/ml (Table 1; Figs. 1-2). Typical cells showed how their surfaces are organised. Since the cytotoxic cells have gotten rounder, smaller, and have shown indications of separation from the surface of the wells, it is likely that the cells have perished (Apoptosis).

Discussion

Fogh and Trempe (1975) successfully separated the HT-29 human colon cancer cell line from a primary tumour collected from a 44-year-old Caucasian female in 1964. Since then, a sizable number of cell lines derived from human colon cancers have been created. This cell line was first used to study different aspects of the biology behind human cancers. However, these cells have drawn a lot of interest since they were able to display traits of mature intestinal cells, such as enterocytes or cells that produce mucus. They are fascinating for a variety of reasons. In order to better understand the molecular processes underlying the differentiation of distinct intestinal cell types, HT29 cells have evolved into a new model. When these cells are cultivated under the proper culture conditions or after being treated with various inducers, such as butyrate or acid, they may be made to demonstrate alternate routes of enterocyte differentiation (Mohanasundaram *et al.*, 2019). The HT29 cell line is regarded as a pluripotent intestinal cell line as a result of this. It is thus appropriate for studying the structural and molecular mechanisms involved in cell differentiation.

There are many advantages to using HT29 as an *in vitro* model of intestinal cells, a list of which may be found in the work of Zweibaum *et al.* (2011). In terms of their shape, the presence of

brush border-associated hydrolases, and the time course of the differentiation process is similar to that seen in the small intestine. This cell line, in its differentiated phenotype, is equivalent to enterocytes seen in the small intestine. Additionally, differentiated HT29 cells display levels of villin that are remarkably similar to those seen in normally developing colonocytes that have just been generated.

The *Coleus forskohlii* extract was tested against the HT-29 cell line at various concentrations (12.5, 25, 50, 100, and 200 µg/ml), and its ability to suppress cell proliferation was observed. The present study demonstrated that the amount of cell growth, that the substance blocked, increased along with its concentration. The study did find, however, that the lowest and maximum concentrations both resulted in growth suppression, with the lowest occurring at 12.5 µg/ml and the greatest occurring at 200 µg/ml. The IC₅₀ value was higher than 195.35 µg/ml. Our results are consistent with previous reports that rutin has cytotoxic effects. Rutin may prevent the development of cancer cells by causing cell cycle arrest and/or death, in addition to reducing proliferation, angiogenesis, and/or metastasis, as shown by Arajo *et al.* (2011) in their research on colorectal cell lines.

Our research indicated that as the extract's concentration rises, the cytotoxic effect gets more apparent. The tetrazolium ring is broken down by the succinate dehydrogenase enzyme, which is only present in live cells, turning the MTT into an insoluble purple formazan. The quantity of formazan produced is directly inversely correlated with the sample's viable cell count (Lee *et al.*, 2004). Some polyphenols may function as xenobiotic-metabolizing enzymes, which affect the metabolic activation of potential carcinogens, to inhibit the growth of cancer cells. Additionally, certain flavonoids may block aromatase and change hormone synthesis to limit the growth of cancer cells (Zhao *et al.*, 2007).

Medical plant extracts may cause target cells to undergo apoptosis in order to exert their cytotoxic

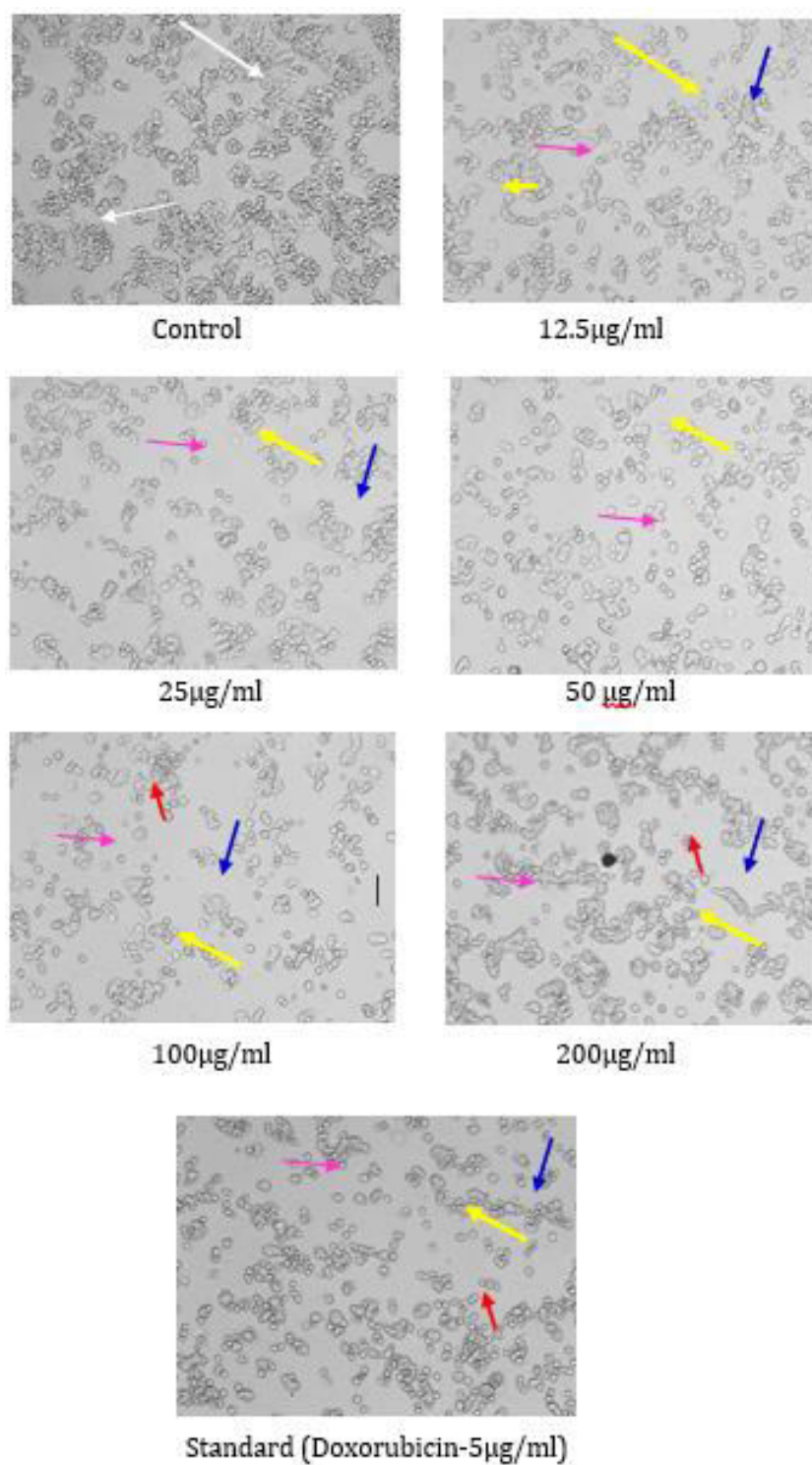


Figure 1: Morphology of HT-29 cell line on different concentrations of *Coleus forskohlii* extract treatment morphology were observed using Inverted at $\times 10$ magnification. The white arrows indicates normal cell, yellow arrows indicates apoptotic bodies, Blue arrows indicates cell debris, red arrows indicates cell shrinkage and violet arrows indicates detached cells

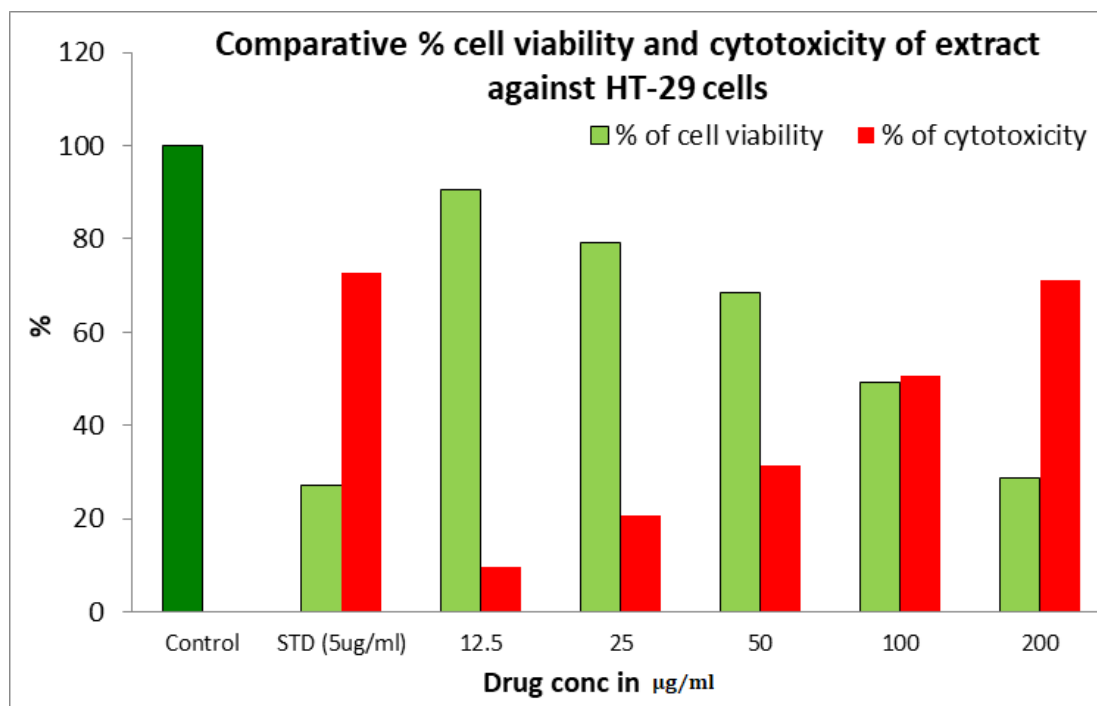


Fig. 2: Comparative % of cell viability and cytotoxicity of extract against HT-29 cells.

effects (Aghbali *et al.*, 2013). Apoptosis, another name for programmed cell death, may occur in both healthy and unhealthy conditions. Cell shrinkage, the presence of apoptotic bodies, cell debris, and detached cells are morphological alterations that help to identify it (Vitalone *et al.*, 2003; Kiss *et al.*, 2006). The results of the present study suggested that the application of the methanolic extract of *Coleus forskohlii* caused the morphological alterations mentioned above. These changes can be a sign that cells that have received treatment have undergone apoptosis. The results showed that the HT-29 cell line was significantly cytotoxic to both the alkaloid and terpenoid present in *Coleus forskohlii* as well as the methanolic extract of *Coleus forskohlii*. The cytotoxic effects of the alkaloid and terpenoid present in *Coleus forskohlii* are substantially more potent than those of the methanolic extract, according to the results of the MTT experiment. Similar findings were reported by Kokhdan *et al.* (2018). The observations of the present study are in conformity with the reports of Sangeetha *et al.* (2019), Ali *et al.* (2020) and Suhas *et al.* (2021).

Through a mechanism that includes interfering with cellular division during the telophase stage of

the mitotic phase, phenolic substances may have an anticancer impact. The quantity of cellular protein, the mitotic index, and the formation of colonies during the process of cell proliferation in cancer cells were also shown to be reduced when *Coleus forskohlii* was used. Another element that contributes to the flavonoid's anticancer activity is the presence of a 4-carbonyl group on the flavonoid's molecule. Additionally, it has been shown that the presence of a 2, 3-double bond in flavonoid molecules is associated with the destruction of mitochondria and the death of cancer cells (Plochmann *et al.*, 2007). This test's main objective was to evaluate the extract's cytotoxicity and establish the toxicity levels in terms of the IC_{50} dosage at a concentration where the proportion of living and dead cells is equal. This is due to the fact that the IC_{50} dosage is thought to be the best dose for each kind of test. It has been shown that the methanolic extract exhibited anti-cancer action at greater concentrations.

The root extract of *Coleus forskohlii* was clearly cytotoxic to the cell line that it was tested on when seen under an inverted microscope. The MTT test, which was used to assess cytotoxicity, was built on the metabolic degradation of MTT. After being

cultivated for 24 h with different concentrations of *Coleus forskohlii* root extract, the morphological changes of the cell lines were compared to the cells that had not been treated. After the incubation time, a considerable difference between the morphology of the control cells and the cancer cells treated with *Coleus forskohlii* was seen. When the extract concentration was reduced, the cells were still viable even though they seemed to be less homogenous and to have lost their membrane integrity. The extract did, however, result in a significant difference between the treated cells and the untreated cells in the control group when utilised at greater doses. The notable changes from untreated cells were the loss of an intact membrane, karyopyknosis, cell detachment from the plate, and modifications to the morphological properties of the cells.

Inverted light microscopy demonstrated the most readily identifiable morphological features of apoptosis in the cells that had been treated with the extract. The treated cells exhibited traits that are typical of cells that are through the apoptotic process. These traits include cytoplasmic condensation, cell shrinkage, nuclear chromatin condensation and aggregation, separation from the culture plate, and loss of interaction with neighbouring cells (Monga *et al.*, 2013).

Conclusion

The results of this study concluded that when tested against a human colon adenocarcinoma cell line, the methanolic extract of *Coleus forskohlii* root had the capacity to suppress colon cancer (HT-29).

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