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Effect of Typhonium flagelliforme Extract on the Viability of Colorectal Cancer Cells HCT-116

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ABSTRACT

Background: Colorectal cancer (CRC) remains a significant global health challenge, with rising incidence rates, particularly among younger individuals [1]. Conventional treatments, including chemotherapy and radiation, often cause severe side effects, necessitating the exploration of alternative therapeutic options. *Typhonium flagelliforme*, a medicinal plant widely used in traditional medicine, has been investigated for its cytotoxic potential against HCT-116 colorectal cancer cells. **Objective:** This study assessed the cytotoxic effects of *T. flagelliforme* extract on HCT-116 colorectal cancer cells using the MTT assay. **Methods:** The cytotoxicity of *Typhonium flagelliforme* (*T. flagelliforme*) extract on HCT-116 colorectal cancer cells was assessed using the MTT assay after 24 hours of treatment with different concentrations (20–100 µg/mL). **Results:** The findings revealed a dose-dependent reduction in cell viability, with an IC_{50} value of 73.47 µg/mL, indicating moderate cytotoxic activity. Higher extract concentrations (100–60 µg/mL) significantly decreased cell viability, while the lowest concentration (20 µg/mL) showed a paradoxical increase, possibly due to a hormesis effect. **Conclusion:** The findings revealed a dose-dependent reduction in cell viability, with an IC_{50} value of 73.47 µg/mL, indicating moderate cytotoxic activity.

Keywords : *Typhonium flagelliforme*, cell viability, colorectal cancer, IC_{50}, cytotoxicity

INTRODUCTION

Colorectal cancer (CRC) is a significant global health concern, ranking as the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths worldwide. In 2022, more than 1.9 million new cases were diagnosed, leading to over 900,000 deaths[1,2]. In the United States, the incidence of CRC has shown a notable shift. While rates have decreased among adults aged 50 and older, there has been an alarming increase among individuals under 50. A recent study reported that CRC cases among younger adults have been rising at an annual rate of approximately 1-2% [3]. This trend underscores the need for heightened awareness and early detection strategies in younger populations.

The rising incidence of CRC among younger populations has been associated with various factors, including genetic predispositions, dietary habits, obesity, and sedentary lifestyles. Emerging research also suggests environmental contributors, such as the consumption of ultra-processed foods, may play a role in this trend. These foods have been linked to increased inflammation and suppressed immune responses, potentially facilitating carcinogenesis [4,5].

Given the increasing prevalence of CRC, especially among younger demographics, there is a pressing need for effective preventive and therapeutic strategies. Traditional treatments, including surgery, chemotherapy, and radiation therapy, often come with significant side effects and may not be effective for all patients. This has led to a growing interest in alternative therapies, particularly those derived from natural sources[6].

Typhonium flagelliforme, commonly known as Keladi Tikus, is a medicinal plant native to Southeast Asia. Traditionally, it has been used to treat various ailments, including cancer. Recent studies have demonstrated its potential anticancer properties across various cancer cell lines. For instance, a systematic review highlighted that *T. flagelliforme* could inhibit cancer cell proliferation, with most IC_{50} values being less than 200 µg/mL. The plant induced apoptosis by increasing caspase-3 and -9 activities and decreasing the expression of the anti-apoptotic Bcl-2 protein. Additionally, it modulated the expression of proteins associated with tumor growth and development, such as p21, tyrosine kinase, Ki67, HER2/neu, telomerase, and COX-2 [7,8].

Furthermore, *T. flagelliforme* contains bioactive compounds like flavonoids, which have shown significant cytotoxic activity against cancer cell lines. These compounds demonstrated high affinity for certain cellular receptors, suggesting a potential mechanism for their anticancer effects[8,9]. This study aims to evaluate the cytotoxic effects of *Typhonium flagelliforme* extract on HCT-116 colorectal cancer cells. Understanding its impact on cell viability could provide insights into its potential as a complementary therapeutic agent for colorectal cancer.

MATERIAL AND METHODS

Research Design

This study aimed to evaluate the cytotoxic effects of *Typhonium flagelliforme* extract on HCT-116 colorectal cancer cells using the MTT assay. A laboratory-based experimental design was applied, with control and treatment groups exposed to varying concentrations of *T. flagelliforme* extract over 24 hours.

Typhonium flagelliforme Extraxtion

Fresh *Typhonium flagelliforme* tubers were collected, washed, and dried at 40°C. The dried samples were ground into a fine powder and extracted using 96% ethanol by maceration for 72 hours at room temperature. The extract was filtered and concentrated using a rotary evaporator to obtain a crude extract. The final extract was stored at 4°C until further use.

Cell Culture and Treatment

HCT-116 colorectal cancer cells were obtained from a cell culture repository (e.g., ATCC) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) or RPMI-1640, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were incubated at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured upon reaching 80-90% confluence.

HCT-116 cells were seeded into 96-well plates at a density of 5×10^4 cells/well and allowed to adhere for 24 hours before treatment. The experimental groups were as follows: HCT-116 cells were seeded into 96-well plates at a density of 5×10^4 cells per well and allowed to adhere for 24 hours before treatment. The experimental groups consisted of three categories. The Blank Control (Control Media) included wells containing only culture media to account for background absorbance. The Negative Control (Control Cells) consisted of HCT-116 cells treated with 0.1% DMSO as a solvent control. The Treatment Groups comprised HCT-116 cells treated with *T. flagelliforme* extract at various concentrations of 10, 25, 50, 100, and 200 µg/mL.

Cytotoxicity Assay (MTT Assay)

After 24-hour incubation, 20 µL of MTT reagent (5 mg/mL in PBS) was added to each well and incubated for 3-4 hours at 37°C. The formazan crystals formed were dissolved using DMSO (Dimethyl Sulfoxide), and the absorbance was measured at 570 nm using a microplate reader. Cell viability (%) was calculated using the following formula:

$$\text{Cell Viability (\%)} = \left(\frac{Absorbance\ of\ treated\ cells\ -\ Absorbance\ of\ media\ control}{Absorbance\ of\ control\ cells\ -\ Absorbance\ of\ media\ control}\right.$$

The IC_{50} value was determined using nonlinear regression analysis in Microsoft Excel.

RESULTS

Cytotoxic Effect of Typhonium flagelliforme Extract on HCT-116 Cells

The cytotoxicity of *Typhonium flagelliforme* (*T. flagelliforme*) extract on HCT-116 colorectal cancer cells was assessed using the MTT assay after 24 hours of treatment with different concentrations (20–100 µg/mL). The results, as presented in Table 1, demonstrate a dose-dependent decrease in cell viability, with higher concentrations of *T. flagelliforme* extract leading to a greater reduction in viable cells. At a concentration of 100 µg/mL, cell viability dropped to 34.78%, indicating a strong cytotoxic effect. When the concentration was reduced to 80 µg/mL, viability slightly increased to 41.60%, though still reflecting a significant reduction in living cells. At 60 µg/mL, the viability remained at 43.57%, suggesting a moderate cytotoxic effect. A weaker cytotoxic response was observed at 40 µg/mL, where cell viability was recorded at 78.36%. Interestingly, at the lowest tested concentration of 20 µg/mL, cell viability increased to 124.67%, suggesting potential cell proliferation rather than inhibition.

The high viability at 20 µg/mL suggests that at lower concentrations, *T. flagelliforme* might have a stimulatory effect on cell growth, known as the hormesis effect. This could be due to low-dose activation of cellular pathways that promote proliferation rather than cytotoxicity

Table 1. Percentage of HCT-116 Cell Viability After 24-hour Treatment

Concentration (µg/mL)	Cell Viability (%)
100	34.78
80	41.60
60	43.57
40	78.36
20	124.67

IC₅₀ Determination

The IC_{50} value, which represents the extract concentration required to inhibit 50% of HCT-116 cell viability, was calculated using nonlinear regression analysis. The IC_{50} for *T. flagelliforme* extract on HCT-116 cells was found to be 73.47 µg/mL. This value suggests that *T. flagelliforme* extract exhibits moderate cytotoxicity against colorectal cancer cells, as cytotoxic agents with IC_{50} values below 100 µg/mL are generally considered to have potential anticancer activity.

The R^2 value of 0.83 indicates a strong correlation between extract concentration and cytotoxic effects, supporting the reliability of the IC_{50} calculation. The relationship between extract concentration and cell viability was analyzed through linear regression. Y represents cell viability (%) and X represents the extract concentration (µg/mL). The coefficient of determination (R^2) = 0.8287, which further confirms that the model fits well with the experimental data. The downward trend in the dose-response curve (Figure 1) indicates that increasing concentrations of *T. flagelliforme* extract correlate with decreased cell viability.

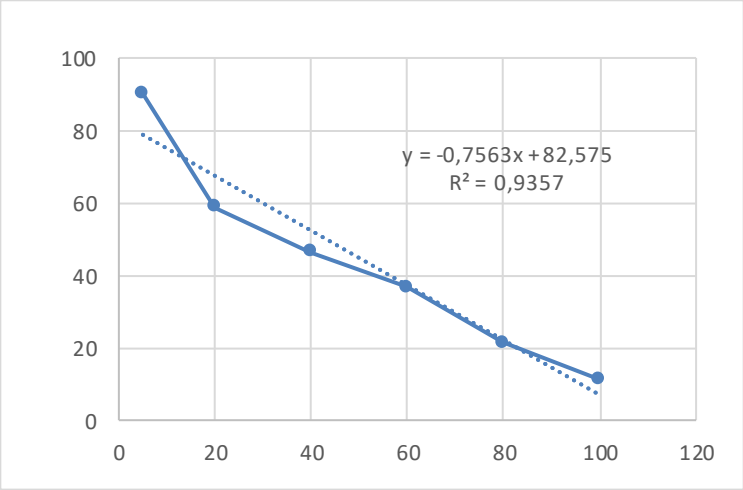

Figure 1. Cytotoxicity Curve of *Typhonium flagelliforme* Extract on HCT-116 Cells.

Interpretation of Cytotoxic Activity

Based on these findings, *T. flagelliforme* extract demonstrates potential cytotoxic effects against HCT-116 colorectal cancer cells, with an IC_{50} of 73.47 µg/mL. The results indicate that at higher concentrations (100–60 µg/mL), the extract effectively reduced cell viability, supporting its potential anticancer properties. In contrast, at the lowest tested concentration (20 µg/mL), an unexpected increase in viability was observed, which could be attributed to hormesis or adaptive cellular responses. The extract demonstrated moderate potency, as indicated by its IC_{50} value, which falls below 100 µg/mL but remains above 50 µg/mL. These findings align with previous studies suggesting that *T. flagelliforme* contains bioactive compounds such as flavonoids, alkaloids, and glycosides, which may contribute to its cytotoxic activity. Further investigations, including apoptosis assays and molecular pathway analysis, are required to confirm the mechanism of action.

DISCUSSION

Typhonium flagelliforme, commonly known as rodent tuber, has gained considerable attention for its anticancer potential due to its diverse bioactive compounds. This study demonstrates that the ethanol extract of *T. flagelliforme* significantly reduces the viability of HCT-116 colorectal cancer cells

in a dose-dependent manner. The cytotoxic effects observed can be attributed to various active compounds present in the plant, which modulate different molecular pathways involved in cancer progression. *T. flagelliforme* contains various bioactive compounds that contribute to its anticancer properties through multiple mechanisms. One of the key components is flavonoids, which possess strong antioxidant and anticancer effects. These compounds inhibit cancer cell proliferation by inducing apoptosis and suppressing angiogenesis, the process of new blood vessel formation that supplies nutrients to tumors, thereby restricting their growth[11,12].

Another important group of compounds found in *T. flagelliforme* is saponins, which increase the permeability of cancer cell membranes, leading to cell lysis and inhibiting further proliferation. Additionally, saponins have been reported to modulate immune responses and enhance the effects of other anticancer agents[13,14]. Similarly, steroids and triterpenoids, such as phytosterols like stigmasterol and β-sitosterol, contribute to the plant's anticancer activity by regulating lipid metabolism in cancer cells, disrupting their membrane integrity, and inducing apoptosis[14]. In addition to these compounds, pheophorbide-related derivatives, including pheophorbide-a, pheophorbide-a', pyropheophorbide-a, and methyl pyropheophorbide-a, exhibit strong antiproliferative activity, particularly in photodynamic therapy. These compounds induce apoptosis and oxidative stress in cancer cells, even in the absence of light activation, making them promising agents for cancer treatment[15]. Furthermore, tannins present in *T. flagelliforme* have been shown to arrest cancer cell cycles at the G2/M phase and promote apoptosis by downregulating anti-apoptotic proteins such as Bcl-2 while upregulating pro-apoptotic markers like Bax[8,9]. Lastly, essential fatty acids, such as linoleic and linolenic acid, play a role in membrane lipid modulation, potentially altering cell signaling pathways and reducing cancer cell viability. These diverse bioactive compounds highlight the potential of *T. flagelliforme* as a natural source of anticancer agents.

The anticancer efficacy of *T. flagelliforme* is closely linked to its ability to modulate key molecular pathways involved in cancer progression. One of the primary mechanisms is apoptosis induction, where flavonoids and pheophorbides activate the intrinsic apoptotic pathway by increasing mitochondrial membrane permeability. This leads to the release of cytochrome c and subsequent activation of caspase-dependent cell death, effectively eliminating cancer cells[16,17]. In addition to promoting apoptosis, *T. flagelliforme* also plays a role in cell cycle arrest. Tannins and flavonoids have been shown to halt cell cycle progression at the G2/M phase, preventing the uncontrolled proliferation of cancer cells and reducing tumor growth potential[10,17]. Another significant mechanism involves PD-L1/PD-1 modulation, where certain bioactive compounds in *T. flagelliforme* may enhance immune responses by downregulating PD-L1 expression in cancer cells. This downregulation increases their susceptibility to immune-mediated destruction, making it a promising candidate for immunotherapy approaches[18]. Furthermore, *T. flagelliforme* contributes to oxidative stress regulation through phytosterols such as stigmasterol, which act as antioxidants. By reducing oxidative stress levels, these compounds disrupt the favorable conditions required for tumor growth and survival, thereby exerting an additional anticancer effect [6]. Collectively, these molecular mechanisms highlight the potential of *T. flagelliforme* as a natural anticancer agent with diverse therapeutic properties.

The present study demonstrated a dose-dependent cytotoxic effect of *T. flagelliforme* extract on HCT-116 cells, aligning with previous reports on the anticancer properties of its bioactive compounds. The observed reduction in cell viability suggests the involvement of apoptosis and cell cycle arrest as key mechanisms. Compared to untreated control cells, those treated with higher extract concentrations exhibited morphological changes indicative of apoptotic cell death, including membrane blebbing and cell shrinkage. Despite promising in vitro findings, further research is required to validate *T. flagelliforme* 's therapeutic potential in vivo and in clinical settings. Standardized extract formulations, toxicity evaluations, and clinical trials will be necessary to establish its efficacy and safety for human

application. Additionally, its combination with existing chemotherapeutic agents should be explored to assess possible synergistic effects.

CONCLUSIONS

This study highlights the potential cytotoxic effects of Typhonium flagelliforme extract on HCT-116 colorectal cancer cells. The results demonstrated a dose-dependent decrease in cell viability, with an IC_{50} value of 73.47 µg/mL, indicating moderate cytotoxic activity. Higher concentrations significantly reduced cell viability, supporting the extract's potential anticancer properties. However, at lower concentrations (20 µg/mL), a paradoxical increase in cell viability was observed, suggesting a possible hormesis effect or adaptive cell response.

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Competing Interests

The authors declare that there is no conflict of interest.

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