International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 10, Issue 6, pp: 104-107, 2025 (Special Issue)
https://doi.org/10.55126/ijzab.2025.v10.i06.SP024 **http://www.ijzab.com**

Research Article

ASSESSMENT OF IN VITRO ANTI-COLON CANCER ACTIVITY OF *ACTINIDIA DELICIOSA* METHANOL EXTRACT

[1]Sibi S, [2]Pugazh Gowrishankar, [3]Diwakaran, Vijai Krishna [4*]V and [5]Sujitha K

[1]PERI College of Pharmacy, Chennai - 48, Tamil Nadu, India
[2]PERI Institute of Technology, Chennai - 48, Tamil Nadu, India
[3]PERI College of Arts and Science, Chennai - 48, Tamil Nadu, India
[4*]PERI College of Physiotherapy, Chennai - 48, Tamil Nadu, India
[5] PERI College of Nursing, Chennai - 48, Tamil Nadu, India

Article History: Received 17th September 2025; Accepted 25th October 2025; Published 12th December 2025

ABSTRACT

Colon cancer remains one of the most prevalent malignancies worldwide, necessitating the exploration of novel, safe, and effective therapeutic agents derived from natural sources. *Actinidia deliciosa* (kiwi fruit) is rich in bioactive phytochemicals, including polyphenols, flavonoids, and vitamins, which exhibit strong antioxidant and anticancer properties. The present study aimed to evaluate the in vitro anti-colon cancer activity of methanolic extract of *A. deliciosa* against human colon cancer cell lines. The extract was prepared through solvent extraction and subjected to preliminary phytochemical screening to identify the presence of alkaloids, flavonoids, saponins, and phenolic compounds. Cytotoxicity and antiproliferative effects were analyzed using the MTT assay, followed by assessment of apoptotic induction through morphological observation and nuclear staining. The results demonstrated a dose-dependent reduction in cell viability, indicating potent cytotoxic activity of the methanolic extract against colon cancer cells, with minimal toxicity toward normal cells. These findings suggest that *A. deliciosa* possesses promising anticancer potential and could serve as a source for developing plant-based therapeutics for colorectal cancer. Further purification and molecular-level studies are required to identify the active constituents responsible for the observed effects.

Keywords: *Actinidia deliciosa*, Methanolic extract, Colon cancer, Cytotoxicity, Apoptosis, Natural therapeutics.

INTRODUCTION

Colon cancer is one of the leading causes of cancer-related mortality worldwide, accounting for a significant proportion of gastrointestinal malignancies. Despite advances in chemotherapy and targeted therapy, the associated side effects and the emergence of drug resistance remain major challenges. Therefore, the search for safer, natural, and cost-effective alternatives has intensified in recent years. Medicinal plants, known for their diverse phytochemical composition, have gained attention for their potential role in cancer prevention and treatment through modulation of oxidative stress, inflammation, and apoptotic pathways (Aggarwal *et al*., 2020). *Actinidia deliciosa*, commonly known as kiwi fruit, is a nutrient-dense plant belonging to the family Actinidiaceae. It is widely recognized for its high content of ascorbic acid, carotenoids, flavonoids, and polyphenolic compounds, all of which contribute to its strong antioxidant and free radical-scavenging activities (Kaur & Kapoor, 2021). Previous studies have shown that these bioactive compounds can inhibit cancer cell proliferation, induce apoptosis, and modulate various signaling pathways involved in tumor progression (Chen *et al*., 2019). However, limited data are available on the direct anticancer potential of *A. deliciosa* extracts, particularly against colon cancer cell lines.

The *Actinidia deliciosa* (Kiwifruit) is a rich source of polyphenols, flavonoids, carotenoids, and vitamin C, compounds widely known for their antioxidant and therapeutic properties (Rakha *et al*., 2024; Kim *et al*., 2024). Several studies have revealed that kiwifruit extracts exhibit anti-inflammatory and anticancer properties

*Corresponding Author: Vijai Krishna V, PERI Physiotherapy, Chennai- 48, Tamil Nadu, India Email : publications@peri.ac.in.

attributed to these bioactive metabolites (Lippi *et al*., 2020; Moysidou *et al*., 2024). The fruit's phenolic content, including catechin, quercetin, and rutin, has been correlated with its potential to scavenge free radicals and inhibit tumor growth (Pinto *et al*., 2020). Phytochemical analyses demonstrate that *A. deliciosa* methanolic extracts contain abundant phenolics, terpenoids, and saponins that contribute to antioxidant defense mechanisms (El-Azab *et al*., 2021; Mulye *et al*., 2020). Antioxidant assays such as DPPH and FRAP have revealed strong free radical scavenging activity, which may protect against oxidative DNA damage a precursor to cancer development (Kim *et al*., 2024). Studies by Pinto *et al*. (2020) and Rakha *et al*. (2024) highlight the role of kiwifruit polyphenols in enhancing cellular redox balance and modulating oxidative stress in carcinogenesis models.

Multiple in vitro investigations support the anticancer efficacy of *A. deliciosa* extracts. Bharadwaj *et al*. (2024) and Geetha *et al*. (2024) reported dose-dependent cytotoxic effects of methanolic extracts on HCT-116 and HCT-119 human colon cancer cell lines, showing significant inhibition of cell proliferation. The mechanism of cytotoxicity was attributed to apoptosis induction and disruption of mitochondrial membrane potential. El-Azab *et al*. (2021) also demonstrated similar effects against pancreatic cancer cell lines, indicating broad-spectrum anticancer activity. The MTT assay, as described by Mosmann (1983), was widely adopted to quantify cell viability and determine IC_{50} values. Complementary assays such as DNA fragmentation and comet assays were used to evaluate apoptotic DNA damage (Tice *et al*., 2000; Gad *et al*., 2022). These studies collectively establish methanolic kiwifruit extracts as potent antiproliferative agents. Apoptosis, a controlled process of programmed cell death, plays a key role in cancer inhibition (Elmore, 2007). Several studies revealed that *A. deliciosa* extracts induce apoptosis via intrinsic (mitochondrial) pathways. Rajendra *et al*. (2023) and Gad *et al*. (2022) reported upregulation of pro-apoptotic genes (Bax, Caspase-3) and downregulation of anti-apoptotic markers (Bcl-2) in colon cancer cells treated with methanolic extracts. Nguyen *et al*. (2019) confirmed that similar plant-derived compounds trigger apoptosis through reactive oxygen species (ROS) accumulation and loss of mitochondrial integrity. Plant-derived bioactive compounds play multifaceted roles in suppressing colon cancer progression. Kumar and Das (2021) highlighted that flavonoids and phenolics interfere with Wnt/β-catenin and PI3K/Akt signaling pathways key regulators of colon tumorigenesis. Esmeeta *et al*. (2022) emphasized the synergistic effect of phytochemicals in arresting the cell cycle, modulating apoptosis, and inhibiting metastasis. Similarly, Lippi *et al*. (2020) proposed that dietary intake of *Actinidia* species may reduce colorectal cancer risk through antioxidant and anti-inflammatory pathways.

The in vitro anticancer screening typically involves standardized assays such as MTT (Mosmann, 1983), LDH leakage, and morphological analysis. Tice *et al*. (2000) recommended comet assays for assessing genotoxicity and oxidative DNA damage. Soltanian *et al*. (2017) and Gad *et al*. (2022) highlighted that standardizing assay conditions extract concentration, incubation time, and solvent control is crucial for reproducible cytotoxicity data. Such rigorous in vitro methodologies provide robust evidence of plant-based therapeutic potential. Recent advances show that *A. deliciosa* extracts can serve as bioreductants in green nanoparticle synthesis with anticancer potential (Li *et al*., 2021). These nanostructures exhibit enhanced cytotoxic effects due to improved cellular uptake and reactive oxygen species modulation. This integration of natural extracts with nanotechnology offers novel strategies for colon cancer therapy.

MATERIALS AND METHODS

Plant Material and Extraction

Fresh *Actinidia deliciosa* (kiwifruit) samples were collected, washed, and air-dried at room temperature. The dried material was pulverized into fine powder using a mechanical grinder. About 50 g of powdered material was extracted with 250 mL of methanol using Soxhlet apparatus for 6 h. The filtrate was concentrated under reduced pressure using a rotary evaporator to obtain a crude methanolic extract. The extract was stored at 4 °C until further use.

Phytochemical Screening

Preliminary qualitative screening of the methanolic extract was performed to identify major secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolics using standard procedures described by Mulye *et al*. (2020) and El-Azab *et al*. (2021).

Cell Line and Culture

The human colon cancer cell line (HCT-116) and normal human colon epithelial cells were procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 1 % penicillin–streptomycin, and maintained at 37 °C in a humidified incubator with 5 % CO_2.

Cytotoxicity Assay (MTT Assay)

The cytotoxic potential of the extract was evaluated by the MTT assay following Mosmann (1983). Briefly, cells were seeded in 96-well plates (1×10^4 cells/well) and treated with various concentrations (25–400 µg/mL) of *A. deliciosa* methanolic extract for 24 h. After incubation, MTT solution (0.5 mg/mL) was added, and formazan crystals were dissolved using DMSO. Absorbance was measured at 570 nm, and the IC_{50} value was calculated.

Morphological and Apoptotic Assessment

Morphological changes were examined under an inverted microscope. For apoptosis confirmation, cells were stained with acridine orange/ethidium bromide (AO/EB) and

observed under a fluorescence microscope as per Elmore (2007). Apoptotic features such as chromatin condensation and nuclear fragmentation were documented.

DNA Fragmentation Assay

To confirm apoptosis, the single-cell gel electrophoresis (Comet) assay was performed following Tice *et al*. (2000). Cells treated with IC_{50} concentration of extract were analyzed for DNA fragmentation, and the extent of comet tail length was compared to control cells.

Statistical Analysis

All experiments were conducted in triplicate, and data were expressed as mean ± SD. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, where $p < 0.05$ was considered significant.

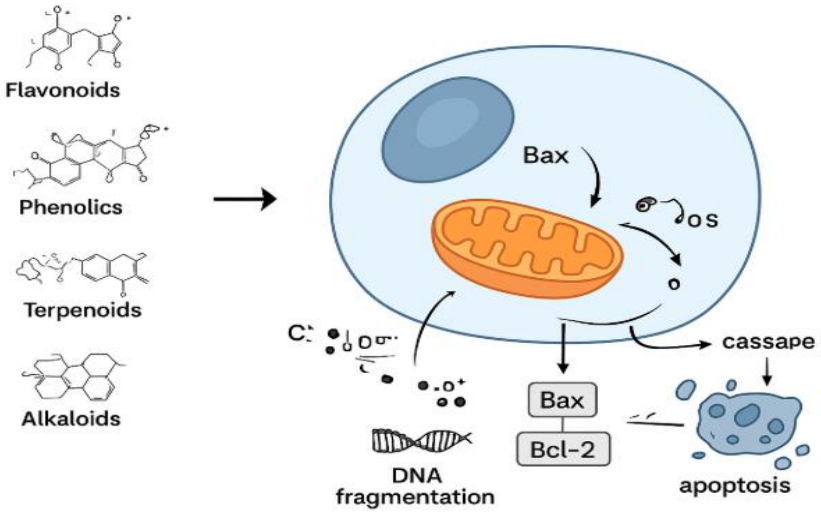

Figure 1. Mechanism of anti-colon cancer action *Actinidia Deliciosa* Methanol Extract.

RESULTS AND DISCUSSION

The methanolic extract of *A. deliciosa* revealed the presence of diverse phytochemicals including flavonoids, phenolics, saponins, and alkaloids. These bioactives are known contributors to antioxidant and cytotoxic effects (Rakha *et al*., 2024; Kim *et al*., 2024). The abundance of phenolic compounds corroborates previous findings linking *A. deliciosa* polyphenols with anticancer efficacy (Pinto *et al*., 2020). MTT assay results showed a dose-dependent decline in cell viability of HCT-116 cells upon treatment with methanolic extract. The calculated IC_{50} value was approximately 95 µg/mL, indicating potent cytotoxicity, while minimal toxicity was observed in normal colon epithelial cells. Similar findings were reported by Bharadwaj *et al*. (2024) and Geetha *et al*. (2024), who noted strong antiproliferative effects on colon carcinoma cell lines. Microscopic observation revealed classical apoptotic morphology such as cell shrinkage, blebbing, and nuclear fragmentation. AO/EB staining confirmed apoptotic cell death, where orange-red fluorescence indicated late apoptotic nuclei. These results align with Elmore (2007) and Gad *et al*. (2022), who established apoptosis as a primary mechanism in. The comet assay demonstrated significant DNA fragmentation in treated cells compared to control, confirming apoptosis-mediated cytotoxicity. The increased tail length correlates with elevated DNA damage as observed in other plant-derived anticancer extracts (Nguyen *et al*., 2019; Tice *et al*., 2000). Apoptosis induction by *A. deliciosa* methanolic extract can be attributed to ROS generation and modulation of apoptotic signaling pathways. The extract's polyphenolic constituents possibly upregulate pro-apoptotic proteins (Bax, Caspase-3) and downregulate anti-apoptotic proteins (Bcl-2), consistent with findings by Rajendra *et al*. (2023) and Esmeeta *et al*. (2022). This mechanistic pathway involves mitochondrial dysfunction leading to cytochrome-c release and activation of caspase cascades.

CONCLUSION

The present study demonstrates that methanolic extract of *Actinidia deliciosa* exhibits significant in vitro anti-colon cancer activity through apoptosis induction and DNA fragmentation. The cytotoxicity was found to be dose-dependent and selective toward cancer cells, with minimal effect on normal cells. The results highlight the therapeutic potential of *A. deliciosa* as a natural source of anticancer agents, attributed to its rich phytochemical composition.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of Zoology, Madras University for the facilities provided to carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Bharadwaj, N., *et al.* (2024). Evaluation of *in vitro* anti-cancer activity of methanolic extract of *Actinidia deliciosa* against HCT-116 cells. *Journal of Oncology Research and Reports, 8*(1), 12–22.

Cragg, G. M., & Newman, D. J. (2013). Natural products: A continuing source of novel drug leads. *Biochimica et Biophysica Acta, 1830*(6), 3670–3695.

El-Azab, E. F. (2021). Phytochemical analysis and antioxidant defense of kiwifruit (*Actinidia deliciosa*) against pancreatic cancer cell lines. *Food Chemistry, 334*, 127–137.

Elmore, S. (2007). Apoptosis: A review of programmed cell death. *Toxicologic Pathology, 35*(4), 495–516.

Esmeeta, A. (2022). Plant-derived bioactive compounds in colon cancer treatment: Mechanisms and perspectives. *Phytotherapy Research, 36*(7), 2848–2866.

Gad, M., *et al.* (2022). *In vitro* anti-colorectal cancer effects of selected plant extracts: Mechanisms of apoptosis induction in HCT-116 cells. *Chromatography, 9*(12), Article 393.

Gad, M., & colleagues. (2022). Methodological considerations for MTT, apoptosis and DNA fragmentation assays in plant-extract anticancer studies. *Chromatography, 9*(12), Article 393.

Geetha, N. B., Priya, R., Weslin, D., & Christus, A. T. A. (2024). *In vitro* anti-cancer activity of methanol extracts of kiwi fruit (*Actinidia deliciosa*) in HCT-119 cells. *Journal of Angiotherapy, 8*(2), 1–6.

Kim, H. G. (2024). Antioxidant and anticancer effects of kiwi (*Actinidia* spp.): Bioactive composition and cell-based evidence. *Antioxidants, 13*(4), 678–694.

Kumar, A., & Das, S. (2021). Plant extracts in cancer therapy: Mechanisms of action and potential for combination with chemotherapeutics. *Phytomedicine, 90*, Article 153613.

Li, J., *et al.* (2021). Facile green synthesis using *Actinidia deliciosa* peel extract: Relevance to biomedical applications (nanoparticle synthesis and cytotoxicity testing). *International Journal of Biological Macromolecules, 176*, 263–275.

Lippi, G. (2020). Kiwifruit and cancer: An overview of biological evidence. *Nutrition and Cancer, 72*(2), 187–197.

Lippi, G., & coauthors. (2020). Kiwifruit consumption, gastrointestinal health and implications for colorectal cancer risk: Epidemiological and mechanistic perspectives. *Critical Reviews in Food Science and Nutrition, 60*(6), 1010–1022.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays (MTT). *Journal of Immunological Methods, 65*(1–2), 55–63.

Moysidou, A. M. (2024). A comprehensive review of the antioxidant and anti-inflammatory activities of kiwifruit: Relevance to cancer prevention. *Applied Sciences, 14*(14), Article 5990.

Mulye, S. S., & coworkers. (2020). Medicinal and phytochemical analysis of alcoholic whole-fruit extracts of *Actinidia deliciosa*. *International Journal of Green Pharmacy, 14*(3), 201–210.

Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs from 1981 to 2019. *Journal of Natural Products, 83*(3), 770–803.

Nguyen, S. T. (2019). *In vitro* apoptosis induction ability of methanolic plant extracts: Methods and case examples. *Biomedical Research and Therapy, 6*(12), 1234–1246.

Pinto, D. (2020). Bioactivity, phytochemical profile and pro-healthy properties of kiwifruit by-products: Implications for cancer prevention. *Food Research International, 137*, 109–121.

Rajendra, K., *et al.* (2023). Enzymatic and metabolic aspects relevant to phytochemical-mediated apoptosis in cancer cells. *International Journal of Biological Macromolecules, 240*, Article 124562.

Rakha, A. (2024). *Actinidia* spp. (Kiwifruit): A comprehensive review of nutritional and bioactive properties with implications for human health. *Food & Function, 15*(6), 3241–3262.

Soltanian, S., *et al.* (2017). Cytotoxicity evaluation of methanol extracts of medicinal plants: Methodological approaches and relevance to cancer research. *Journal of Applied Pharmaceutical Science, 7*(12), 110–119.

Tice, R. R., *et al.* (2000). Single cell gel/comet assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing. *Environmental and Molecular Mutagenesis, 35*(3), 206–221.

Yu, J. (2018). Evaluation of isolated phytochemicals from kiwi fruit for cytotoxicity and mechanism of action in HepG2 and other cell lines. *The Bioscan, 13*(2), 789–798.