In vitro Anti-cancer Activity of Methanol Extracts of Kiwi Fruit, *Actinidia deliciosa* in HCT-119 Cells

Geetha N B [1], Priya R [1], Weslin D [1], Ashmi Christus A T [2]

Abstract

This study determined the potential of kiwi fruit (*Actinidia deliciosa*) to inhibit cancer cell growth on HCT-119 cells. We used MTT and COMET assays to assess its biological activity. The kiwi extract exhibited cytotoxicity against these cells, with an IC50 value of 30.038 mg/ml. Microscopic examination revealed damage to cell membranes, reduced adhesion, and inhibited cell growth. Fluorescence staining indicated changes in cell nuclei, suggesting apoptosis. Moreover, the extract appeared to halt HCT-119 cell growth by activating specific cell death pathways. These findings imply that kiwi extract holds promise as a treatment for colon cancer due to its beneficial compounds. We advocate for further clinical studies on kiwi fruit extract to explore its potential in cancer therapy.

Keywords: *Actinidia deliciosa*, HCT-119 cells, MTT assay, COMET assay, kiwi fruit extract.

Significance | The study of anti-cancer properties of Actinidia deliciosa methanol extract in Colorectal cancer as effective treatments.

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Introduction

Cancer is a major cause of death globally, with colorectal (CC) cancer being prominent. It ranks third in men and second in women for cancer diagnoses annually, with over 1.4 million new cases per year (Wu et al., 2002; Kuttan et al., 1985; Solowey et al., 2014;, Yin et al., 2004; Devi et al., 2012; Kajani et al., 2014). While more than half of CC cases occur in developing countries (Yusefi et al., 2020), mortality rates are higher due to limited resources (Rehana et al., 2017; Gomathi et al., 2020; Naya et al., 2015). Conversely, mortality rates have decreased in Western countries with improved early diagnosis and CC care (Dadashpour et al., 2018; Kosanić et al., 2012). Cancer cell survival involves decreased apoptosis, making it a target for chemotherapy (Solowey et al., 2014; Rehana et al., 2017; Lichota & Gwozdzinski, 2018; Al-Rimawi et al., 2016; Colic & Pavelic., 2000). Apoptosis induction is a key aspect of traditional cancer treatments, including chemotherapy, radiation, and hormonal therapies (Fouché et al., 2008; Lakshmanan et al., 2018; Chanda & Nagani, 2013).

It's crucial to urgently evaluate the reasons behind the low survival rates of colorectal cancer (CC) patients. Conducting a benchmark analysis is essential for formulating effective policies to address the disease in India, allowing for an assessment of the demographic and clinical profiles of CC patients (Chanda & Nagani, 2013; Nguyen et al., 2020; Shoemaker et al., 2005). Oncologists in India observe that CC predominantly affects younger generations, often presenting with advanced-stage diseases, signet ring morphologies, and a higher prevalence of anorectal sites compared to global trends (Patil & Kim, 2017; Kiran et al., 2018; Yusefi et al., 2020).

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Some research on colorectal cancer (CC) patients in India exists, but these studies often have small sample sizes, which limits the validity of their conclusions (Kosanić et al., 2012). Additionally, none of these studies analyzed a substantial number of CC patients to provide comprehensive insights into demographic and therapeutic aspects (Voss et al., 2006).

In our daily diets, fruits play a crucial role as they are low in calories, salt, fat, and cholesterol, while being rich in flavor and easily digestible. They are packed with antioxidants, enzymes, and minerals, which can help reduce the risk of many diseases (El-Hallouty et al., 2015).

Over the past two decades, kiwi fruit has gained popularity due to its numerous medicinal properties. Also known as the Macaque and Mihoutau peach (Ferguson & Seal, 2008), it is believed to originate from the Chang Kiang Valley in northern China, hence its other name, "Yang Tao" (Ferguson, 1984). Originally cultivated in mountainous, forest-based regions of China, the kiwi plant is a robust, leaky climber that tolerates full sun or semi-shade (Talens et al., 2002).

Kiwi fruit, typically about 3 inches long, has a brown hairy peel and white pulp with numerous minute, black edible seeds in the middle. It is versatile and can be used to make various products such as dried kiwi, frosted kiwi, honey, jelly, jam, juice, and nectar (Jordan et al., 2002; Huang et al., 2013; Duttaroy & Jørgensen, 2004). In some cultures, kiwi fruit juice is commonly used as a tenderizer (Jordan et al., 2002; Huang et al., 2013; Duttaroy & Jørgensen, 2004).

Kiwifruit is packed with phytonutrients, essential vitamins, and minerals that promote health. It contains high levels of glucose and fructose, along with a small amount of saccharose (Park et al., 2014). Rich in antioxidants like beta carotene, lutein, Xanthine (vitamin A), vitamin E, and vitamin K, kiwifruit offers various health benefits (Richardson et al., 2018). Studies have shown that regular consumption of kiwifruit may be beneficial for conditions like asthma and diabetes (Jaeger et al., 2003).

Moreover, kiwifruit is a potent source of potassium, even more so than bananas or citrus fruits. It also contains several medically important substances that can aid in improving sleep quality (Iwasawa et al., 2011). Certain varieties of kiwifruit found in China exhibit strong anti-mutagenic effects and can boost immune function.

To enhance the health benefits of kiwifruit, efforts are being made to isolate and utilize its effective components, such as anti-cancer isoflavones, organic acids, polysaccharides, and trace elements, in the development of medications or drinks (Ferguson, 2014).

This study aimed to explore the anti-cancer potential of methanol extract from Actinidia deliciosa. The anti-colorectal cancer activity of the extract will be evaluated through cytotoxicity assays, morphological analysis, assessment of adhesive cell properties, and induction of apoptosis. Given its remarkable medicinal potential, Actinidia deliciosa holds significant conservation value.

Materials and Methods

Collection and authentic identification of plant species

A. deliciosa fruits were purchased from Koyambedu fruit market, Chennai, Tamil Nadu, India and were authentically identified by Dr. P. Jayaraman, PARC, West Tambaram, Chennai, India.

Extraction of *A. deliciosa* fruits

Extraction of *A. deliciosa* fruits using METH extracts was done according to the method of Ashok and Sivakumari (2020). In July 2020, *Actinidia deliciosa*, commonly known as kiwifruit, was obtained from the local market. The fruit was processed by separating the flesh from the skin and blending it using a high-speed hand blender. Methanol extracts were prepared by mixing the flesh or peels with 70% ethanol in a 1:1 ratio for 24 hours at 30°C. The resulting filtrates were concentrated using a rotary vacuum evaporator and then lyophilized before being stored at -80°C until further use. The lyophilized powder was dissolved in 70% ethanol for subsequent experiments.

Cytotoxicity Study

HCT-119 cells were procured from NCCS Pune, India. MTT assay was followed to assess the viability of human CC cell line (HCT-119) using different solvent extracts of *A. deliciosa*. Following the method described by Ashok and Sivakumari (2020), the anti-cancer activity was assessed using the MTT assay. HCT-119 cells were seeded onto 96-well plates at a density of 1 x 10^5 cells/ml (100 µl per well), with sterile phosphate buffer saline (PBS) added to the edge as a blank control. After incubating at 37 °C with 5% CO2 for one day to allow cell attachment, the medium was removed, and the monolayer cells were washed twice with 1 ml of trypsin (0.25%)/EDTA (0.05%) solution when reaching full confluence. The sample extract was diluted in DMEM medium (containing 2% serum), with 0.1 ml of each dilution pipetted into the wells, while the control wells contained only DMEM medium. After incubation at 37 °C, the plates were examined for signs of toxicity. Subsequently, 20 µl of MTT (5 mg/ml PBS) was added to each well and incubated for 1-5 h at 37 °C in 5% CO2. To dissolve the formazan (MTT metabolic product), 200 µl of DMSO was added to each well and stirred. The absorbance at 560 nm was then recorded using a microplate reader (MR-96A, Mindray, China). The survival cell percentage was calculated using Equation 1.

$$\%\text{Cell viability} = \frac{\text{A treated cell}}{\text{A control cells}} \times 100 \qquad \text{----------(1)}$$

PI staining assay

PI assay was done according to the method of Ashok and Sivakumar (2020). To conduct the cell viability analysis using propidium iodide (PI), 5ml Falcon polypropylene Round-Bottom test tubes, a

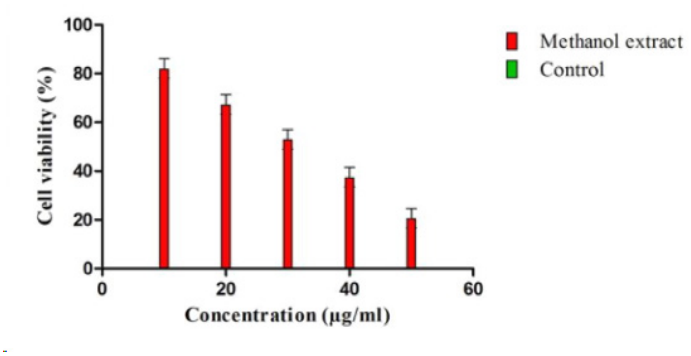

Figure 1. Cell viability of Actinidia deliciosa methanol extract treated with HCT-119 cells

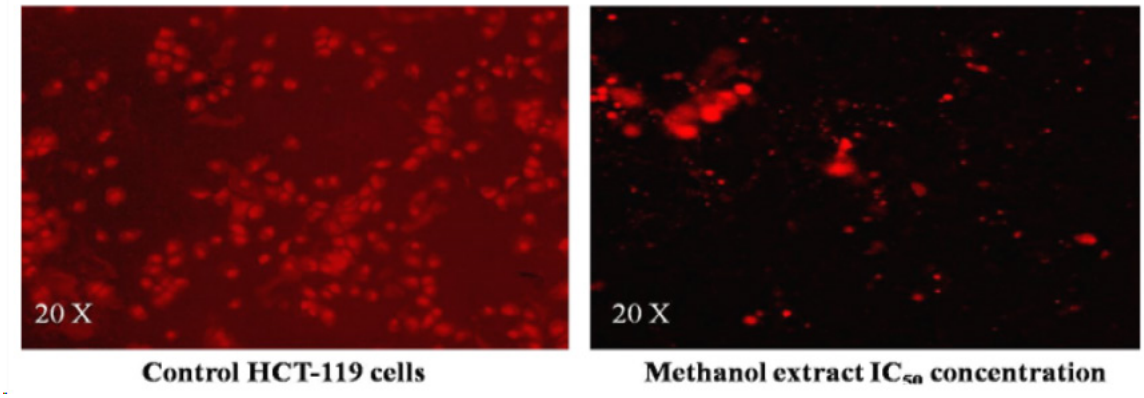

Figure 2. PI staining of HCT-119 cells

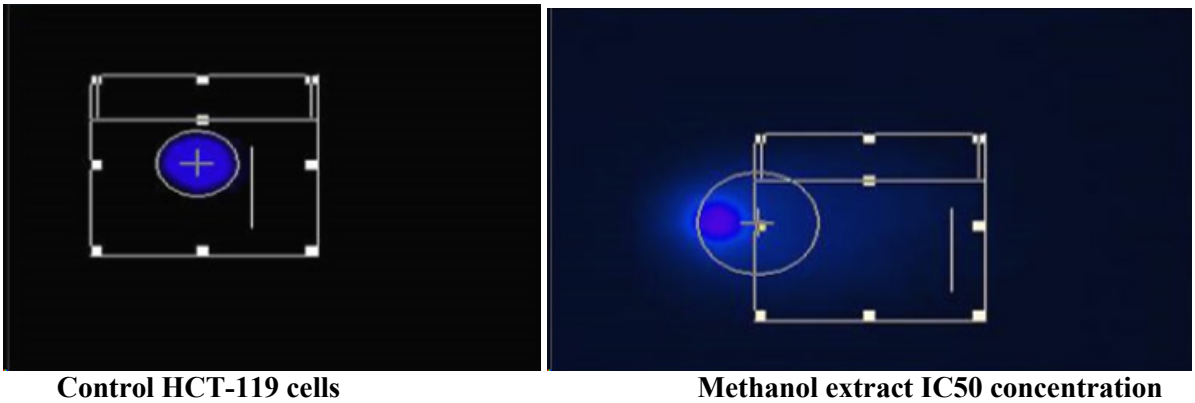

Figure 3. COMET assay of HCT-119 cells

cooling centrifuge, sterile-filtered Phosphate-buffered saline (PBS), and PBS with either 2% FBS or 0.1% BSA were used. A 1 x 10^6 cells/ml cell suspension and Propidium iodide (PI) solution, was prepared by dissolving PI (Sigma, P 4170 or Invitrogen # P3566) in dH2O at a concentration of 1 mg/ml. Cells were trypsinized for 10 minutes at 37°C, with serum. The cell suspension was washed twice with PBS + 2% FBS, and then resuspended and aliquoted as 1 x 10^6 cells in 1 ml PBS + 2% FBS. A final concentration of 2 µg/ml PI was added to the cell suspension. Cells were incubate for 15 minutes on ice in the dark, and analyzed using a flow cytometer.

COMET Assay

assay was done according to the method of Ashok and Sivakumar (2020). In brief, around 12,000 cells mixed with low melting point agarose were placed on microscope slides coated with normal melting point agarose. After setting, the slides were processed in batches using a high-throughput slide rack. Following incubation in a lysis buffer and electrophoresis, the cells were neutralized and washed. Slides were then dried overnight and stained with propidium iodide solution. All steps were conducted under red light to minimize DNA damage. The comets were observed using fluorescence microscopy, and Comet Assay IV software was used for analysis. Each experiment was replicated at least twice.

Statistical analysis (StA)

The data were evaluated using 2-Way ANOVA. Statistically important, the $p < 0.05$ was identified. Five independent studies show the findings as mean ± S.D.

Results and Discussion

An MTT test was conducted on colorectal cancer cells using *Actinidia deliciosa* methanol crude extract (HCT-119) to evaluate its cytotoxic properties. Figure 1 depicts the cytotoxic activity of the methanol extracts, indicating significant cytotoxicity against HCT-119 cells at a concentration of 30.038 mg/ml. This analysis highlights the potential of Actinidia deliciosa methanol extract as a novel anti-cancer agent, supported by its effectiveness in MTT tests. The study also underscores the medicinal significance of plants like Actinidia deliciosa, which have been traditionally used in folk and herbal medicines, offering promising prospects for modern pharmaceutical drug development (Ashok and Sivakumari 2020; Fu et al 2020; Graziani et al 2018; Wang et al 2020; Aires & Carvalho 2020). The cytotoxicity might be the reason of bioactive compounds, such as polyphenol, have been observed for the methanol extracts of this plant for biological activities (Brunetti et al 2020, Wang et al 2018, Kim et al 2020). These compounds are found in many food products and have considerable potential as pharmaceutical candidates because of their efficacy, low toxicity and broad public acceptance.

For further analysis, we determined the mechanism of *Actinidia deliciosa* methanolic extract using PI staining and Comet assay with its IC50 30.038 mg/ml (Figure 2). Microscopy tests revealed apoptotic activity, such as chromatin condensation and nuclear fragmentation, in HCT-119 cells treated with this IC50 concentration compared to control cells, indicating potential anti-cancer activity. Additionally, our findings support the notion that methanol extracts play a role in DNA damage repair, with evidence suggesting early-stage DNA damage induction in HCT-119 cells. The Comet assay confirmed methanol extract-induced DNA damage in IC50-treated cells compared to controls (Figure 3). Methanol extract also influenced programmed cell death by modulating the Bax to Bcl-2 ratio, as observed in previous studies and corroborated in our research involving DU145 and LNCaP prostate cancer cells (Peng et al 2019, Ashok and Babu, 2019) . This highlights the significance of the Bcl-2 family in regulating the intrinsic pathway of apoptosis.

Conclusion

In conclusion, the methanol extract of *Actinidia deliciosa* demonstrates promising anti-cancer and anti-apoptotic effects against HCT-119 cells in vitro. This highlights the potential therapeutic value of kiwi fruit, promoting its increased recognition and utilization for public health purposes. The identification of active phytochemicals in kiwi fruit underscores its natural cytotoxic properties against various cancers. This research suggests that exploring and harnessing the therapeutic potential of kiwi fruit could lead to the development of novel anti-cancer agents with broader applications in cancer treatment and prevention.

Author contribution

G.N.B., P.R., W.D., A.C.A.T. conceptualized, reviewed the literature, and wrote the article.

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None declared

Competing financial interests

The authors have no conflict of interest.

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