In vitro Cytotoxicity and Apoptotic Activity of Aquous, Chloroform and Methanol Extracts of Kiwi Fruit *Actinidia deliciosa* in MCF-7 Cells

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Abstract

The study aimed to assess the anti-cancer potential of *Actinidia deliciosa* Aquous (AQU), Chloroform (CHL) and Methanol (METH) extract on the MCF-7 cell line. MTT cytotoxicity assay was used to determine the anti-cancer activity of the fruit extracts. DAPI was also used to determine the apoptotic cell imaging. The MTT assays revealed dose-dependent cytotoxic effects, with METH extract exhibiting the highest potency, with an IC50 value of 70.984 µg/ml after 24 hours of incubation. DAPI staining confirmed apoptotic cell death induced by the extract. The observed cytotoxic and anti-cancer effects are likely attributed to bioactive compounds in *A. deliciosa* fruit extracts. These findings suggest the potential of *A. deliciosa* METH extract as a natural source for cancer medication development. Further investigation is needed to identify the active compounds responsible for these effects and their apoptotic pathways. Future research should explore its efficacy in vivo and its potential as a dietary supplement or therapeutic agent.

Keywords: *Actinidia deliciosa*, Kiwi fruit (KF), MCF-7 cell line, MTT, DAPI staining.

Significance | Assessment of A. deliciosa extracts' anticancer potential on MCF-7 cells offers insights for natural cancer medication development

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Introduction

Breast cancer (BC) remains a significant global health concern, with mortality rates projected to rise sharply by 2040. While chemotherapy is a common treatment, its side effects and uncertain efficacy prompt the exploration of natural remedies like kiwifruit (KF). This study aims to investigate KF extracts' potential in combating BC cell proliferation and death processes.

Breast cancer (BC) stands as the second-leading cause of death globally, posing a significant health threat. Research by Chung and Gillison (2009), Demark-Wahnefried et al (2012), and others underscores its growing concern. The International Cancer Research Agency (IARC) reports alarming mortality rates and diagnoses (Moore et al., 2010). By 2040, projections suggest a staggering increase in BC-related deaths, reaching 6.99 million (**Mazza**, 2004).

Epidemiological surveys highlight the rising incidence of BC in both developed and emerging countries (Arbyn et al., 2020). Despite significant research efforts, chemotherapy remains the primary treatment option, as discussed by Halkidou et al. (2003) and Moore et al. (2010). However, the efficacy of chemotherapy remains uncertain, with risks and side effects ranging from life-threatening to short-term (Moore et al., 2010).

The limitations of chemotherapy stem from its lack of specificity to cancer cells, leading to adverse effects (Jiao et al., 2020). This highlights the need for alternative treatments, especially considering the reliance of nearly eighty percent of the population on natural health services (Vilchez and Butel, 2004).

Recent studies have linked regular consumption of nutrient-rich fruits like kiwifruit (KF) to improved cardiovascular, immune, and digestive health (Mesfin et al., 2009; Richardson et al., 2018;

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Singletary, 2012). KF is particularly notable for its high vitamin C content, dietary fiber, potassium, and antioxidants, offering numerous health benefits.

The production of vegetable chemicals like Actinidine, which stimulates motility, presents a promising avenue for enhancing health (Ferguson and Ferguson, 2002). Various cultivars of Actinidia species, such as Hayward (green KF) and A. chinensis (gold KF), offer diverse flavors and textures, catering to different preferences (Stonehouse et al., 2013; Hunter et al., 2016; Beverland, 2001).

The objective of this research is to study the cytototoxic capabilities and the potential death processes of CA cell-centered cells of human BC cells, such as aqueous (AQU), chloroform (CHL) and methanol (METH) extraction of *A. deliciosa* fruit (MCF-7).

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 AQU, CHL, and METH extracts was done according to the method of Ashok and Sivakumari (2020) and Latocha et al (2015). 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. Aqous extract was prepared with 100% water. Chloroform and 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

MCF-7 cells were procured from NCCS Pune, India. MTT assay was followed to assess the viability of human BC cell line (MCF-7) using different solvent extracts of *A. deliciosa*. Following the method described by Mosmann (1983) and Ashok and Sivakumari (2020), the anti-cancer activity was assessed using the MTT assay. MCF-7 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.

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

Statistical analysis (StA)

The MTT assay 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

A. deliciosa fruit extract's cytotoxic ability against the human BC cell line (MCF-7) is still not investigated. This research has thus been performed to examine the cytotoxic in vitro effects of various fruit extracts, *i.e.* AQU, CHL and METH extracts of *A. deliciosa* against human BC cell line (MCF-7). ANC activities were evaluated using MTT and DAPI staining methods, as well as apoptotic morphologic analyses of the AQU, CHL and METH extracts. This study employed MTT and DAPI stained tests because they are simplistic, accurate, adaptive, and used to measure fruit extracts' cytotoxicity and ANC effects.The IC_{50} values of test extracts were calculated, and the results are tabulated in Table 1. The IC_{50} of different solvent extracts was recorded as 89.807 µg/ml (AQU), 106.709 µg/ml (CHL) and 70.984 µg/ml (METH), respectively. Based on the results, METH extract showed the best activity when compared to different solvent extracts. Further, these concentrations were tested by DAPI staining to confirm cell apoptosis. Henceforth, the METH extract IC_{50} concentration could be used in the ANC activity.

DAPI staining of MCF-7 cells was performed by fluorescence microscopy study to decide if the growth inhibitions of MCF-7 cells are related to cell mortality (Figure 1). The untreated (Control) MCF-7 cells revealed many viable cells and a reduced number of apoptotic cells found for *A. deliciosa* extracts after 24 hours of incubation (Figure 2), based on morphological description. Normal cell death can be caused by apoptotic cells in untreated MCF-7 cells. This can be due to higher cell density, lower cultivation of nutrient solutions, increased metabolism, cell loss resulting from nutritional effects and a cessation of the proliferation of metabolised substances. Dissemination was reduced for the treated cells in *A. deliciosa* extract in dose-dependent comparison to apoptotic cells. In particular, the differentiation of the apoptotic bodies and the treatment's bluish coloring with DAPI attachment to damaged DNA was observed at 24 h. Although *A. deliciosa* methanol revealed many necrotic cells, also at low concentrations, with cell viability reductions. Because of the findings, only apoptotic

Table 1: MCF-7 cell viability for 24 hours in KF extracts

Concentration	AQU	CHL	MET
25 µg/ml	84.47± 0.402 (-15.53)	92.796±0.537 (-7.204)	80.724± 0.405 (-19.276)
50 µg/ml	72.578±0.295 (-27.422)	85.624±0.609 (-14.376)	65.058±0.058 (-34.942)
75 µg/ml	59.638±0.878 (-40.362)	75.13±0.206 (-24.87)	49.484±0.562 (-50.516)
100 µg/ml	45.582±0.303 (-54.418)	59.212±0.261 (-40.788)	32.084±0.119 (-67.916)
125 µg/ml	31.282±0.490 (-68.718)	40.42±0.389 (-59.58)	13.91±0.737 (-86.09)
IC_{50}	89.807	106.709	70.984

Values are mean ± SE of five individual observations
Values in parenthesis are percent change in compared to control
Denotes percent decrease over control
**values are significant at P<0.05*

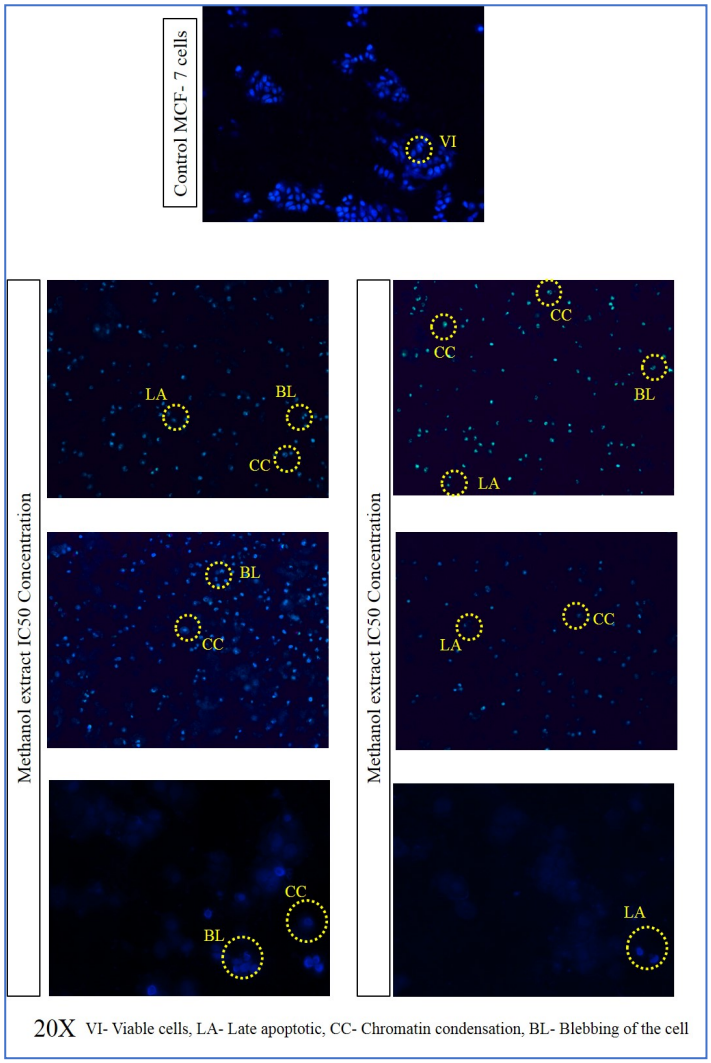

Figure 2. DAPI staining of MCF-7 cells for 24 h

characterizations in MCF-7 cells that maintain cell viability, morphology of apoptosis, and reduced amount of necrotic characteristics were promoted by *A. deliciosa* METH extract. We therefore examined the efficacy of METH extract in ROS production. The sensitivity to these extracts greatly improved intracellular ROS at all dose-based levels in the MCF-7cells.

Due to different solvents of polarity, this difference is probably due to the METH polarity of AQU and CHL and some unique molecules that delivered their cytostatic function MCF 7 cells in our sample, which contain the phytochemicals of the METH extract. Therefore it may be inferred to be polar compounds found in METH excerpts for the differential ANC behaviours of *A. deliciosa* observed in this report. Our findings also agree with the other studies that various plant extracts have cytotoxic effects on MCF-7 by Engel et al. (2014), Khateef et al. (2019), Sarli and Ghasemi (2020), García-Solís et al. (2009) and Gomathi et al. (2020). CA has evolved several regulated growth escape mechanisms that prevent apoptosis. Therefore, the use of KF METH extract containing multiple compounds with potential intracellular objectives may be advantageous over the use of the single KF compound. Since most plants tested have a very long history of oral use, particularly *A. deliciosa*, and tend to be non-toxic, the capacity to cultivate whole KF extracts in conjunction with other medicines alone or in addition to the regular diet as a preventive medication is encouraging. However, these berries, including further experiments in vivo in animal models and human clinical trials, should also be reviewed before any particular human application for CA.

Conclusion

Based on these observations, it is obvious that the extract of *A. deliciosa* METH extract can be considered an essential ANC source. METH extract from *A. deliciosa* was found to have substantial ANC activity using MCF-7 cell line. Therefore, polyphenols could be responsible for *A. deliciosa's* ANC potential. He will also provide support as an ideal actor for bio-pharmaceutical therapeutic agents and dietary supplement products. Prospects for conducting an in vivo analysis and exploring *A. deliciosa* as a replacement for the synthetic flavour enhancer could be a positive move forward.

Author contribution

K., A.C., A.K., S.M. conceptualized, reviewed the literature, and wrote the article.

Acknowledgment

None declared

Competing financial interests

The authors have no conflict of interest.

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