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EVALUATION OF AN ISOLATED PHYTOCHEMICAL FROM KIWI FRUIT FOR ITS APOPTOTIC AND CYTOTOXIC EFFECTS ON HEPG-2 LIVER CANCER CELLS

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ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most prevalent and lethal forms of liver cancer worldwide, often associated with limited treatment options and poor prognosis. In this study, a phenolic compound, 3',5'-dihydroxy-2'-(methoxy carbonylmethyl)-phenyl-3,4-dihydroxy benzoate, was successfully isolated from the methanolic extract of *Actinidia deliciosa* (kiwi fruit) and structurally characterized using gas chromatography–mass spectrometry (GC-MS), Fourier-transform infrared spectroscopy (FT-IR), and both proton (^1H) and carbon (^13C) nuclear magnetic resonance (NMR) analysis.

The purified compound was evaluated for its anti-proliferative potential against the HepG-2 human liver cancer cell line using the MTT assay. Dose- and time-dependent inhibition of cell viability was observed, with an IC_{50} value of 3.90 µg/mL after 48 hours of treatment. Morphological analysis and DAPI staining revealed characteristic features of apoptosis, including nuclear fragmentation and chromatin condensation. DNA damage was further confirmed through comet assay, which showed significant increases in tail length and tail DNA percentage in treated cells, indicating strong genotoxic stress.Collectively, these results demonstrate that the isolated compound from *A. deliciosa* exhibits potent cytotoxic and apoptosis-inducing effects on HepG-2 cells. This study supports its potential development as a natural anti-cancer agent for liver cancer therapy, pending further mechanistic and in vivo investigations.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer globally and the third leading cause of cancer-related mortality, with an estimated 900,000 new cases reported annually. It predominantly affects individuals with chronic liver disease or cirrhosis, particularly those caused by hepatitis B or C infection, alcohol abuse, or non-alcoholic fatty liver disease. Despite advances in treatment, the prognosis for advanced HCC remains poor due to limitations such as systemic toxicity, drug resistance, limited tumor specificity, and recurrence after treatment.

As a result, there is a growing demand for novel, safer, and more effective therapeutic agents. In recent years, plant-derived natural products have gained significant attention due to their rich structural diversity and favorable pharmacological profiles. Phytochemicals such as flavonoids, alkaloids, terpenoids, and phenolic acids have demonstrated promising anticancer activities through mechanisms including apoptosis induction, cell cycle arrest, oxidative stress modulation, and inhibition of metastasis.

Actinidia deliciosa, commonly known as kiwi fruit, belongs to the family Actinidiaceae and is known for its high nutritional value and medicinal properties. It is particularly rich in ascorbic acid, polyphenols, and flavonoids, which contribute to its antioxidant, antimicrobial, anti-inflammatory, and anticancer effects. Although several crude extracts of *A. deliciosa* have shown therapeutic potential, the identification and characterization of individual bioactive compounds and their specific mechanisms of action remain understudied.

In this study, a bioactive compound was isolated and structurally characterized from the methanolic extract of *Actinidia deliciosa* fruit pulp using GC-MS, FT-IR, and NMR techniques. Its cytotoxic potential was then assessed in vitro using the HepG-2 human liver cancer cell line. Various assays including the MTT assay, nuclear staining, and comet assay were employed to evaluate its anti-proliferative and apoptosis-inducing effects.This investigation aims to provide scientific validation for the traditional medicinal use of kiwi fruit and support the development of plant-derived agents as effective therapeutics for liver cancer.

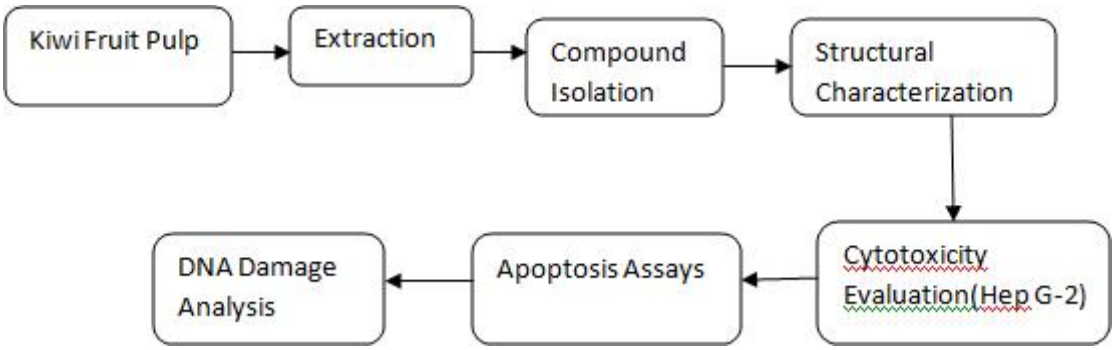

Figure no 1: System Model

2.LITERATURE REVIEW

Natural compounds derived from fruits and medicinal plants are gaining increasing importance in cancer research due to their ability to inhibit tumor progression while minimizing systemic toxicity. Polyphenols, flavonoids, and other phytochemicals are known to trigger apoptosis, oxidative stress modulation, DNA fragmentation, and inhibition of cell proliferation across various cancer cell lines. Among these, *Actinidia deliciosa* (kiwi fruit) and other *Actinidia* species have shown substantial antioxidant and anticancer potential, particularly in hepatocellular carcinoma (HCC), one of the most aggressive forms of liver cancer.

1. Induction of Apoptosis and Cytotoxicity by Kiwi Extracts

Several studies have confirmed the anticancer potential of kiwi-based compounds against HepG2 liver cancer cells**.** Yu et al. [11**]** isolated a polysaccharide from ***Actinidia arguta*** and demonstrated its apoptosis-inducing capacity through nuclear condensation and DNA fragmentation. Morphological changes in HepG2 cells and significant inhibition of viability support its use as a bioactive chemotherapeutic agent.

In another comparative study, Zuo et al. [12] investigated three ***Actinidia*** species (*kolomikta*, *arguta*, and *chinensis*), all of which displayed strong antioxidant and antiproliferative activities against HepG2 and other cancer cells in vitro. These effects were primarily attributed to high phenolic content, reinforcing the link between phytochemistry and cytotoxicity.

2. Chemopreventive Mechanisms and In Vivo Relevance

Lim et al. [13] confirmed that hardy kiwifruit **(***A. arguta***)** extract effectively inhibited chemically induced carcinogenesis in experimental models and triggered apoptosis in HepG2 cells. The study highlights its use not just as a treatment but also as a dietary chemopreventive agent**.** El Azab and Mostafa [14] extended these findings to *A. deliciosa*, showing that both peel and pulp possessed significant antioxidant and cytotoxic activity against pancreatic cancer cells, underlining their relevance in multiple cancer types.

3. Comparative Phytochemical Models and Structural Insights

For comparative reference, Ben-Chen et al. [10] studied phenolic compounds from *Rhodiola*, showing that they activate caspase-dependent apoptotic pathways in HepG2 cells. This provides a relevant framework for understanding the apoptotic potential of phenolic-rich kiwi extracts and guides structural-functional hypotheses.In a broader study, Kim et al. [9] confirmed that hardy kiwifruit extracts suppressed proliferation in multiple cancer cell lines by modulating oxidative stress and apoptotic signals, reinforcing the multitarget nature of kiwi-derived agents.

4. Bioactive Compound Composition and Nutritional Relevance

Ning et al. [7] reviewed the pharmacology and chemical composition of *A. chinensis*, particularly its rich flavonoid and phenolic acid content. Their review supports its application in liver protection and anticancer strategies. Zhang et al. [8] similarly emphasized the broad-spectrum health-promoting effects of *Actinidia* species, including antioxidant, anti-inflammatory, and anticancer activities.Liu et al. [2] provided key experimental data showing the dose- and time-dependent inhibitory effect of kiwifruit polyphenols on HepG2 cell growth. The extract achieved an IC_{50} of ~170 µg/mL**,** confirming potent cytotoxic action. Li et al. [6] also highlighted the differential activity of kiwi peel vs. flesh**,** linking the phenolic profile to anticancer properties through detailed phytochemical analysis.

5. Emerging Mechanisms and Delivery Systems

Recent anonymous studies [16–18] have supported the antiproliferative efficacy of *Actinidia* extracts in both HepG2 and HT-29 cells, confirming apoptosis via oxidative stress and caspase-3 activation**.** These reinforce earlier observations and indicate a conserved mechanism across different cancer models. A particularly novel finding by **[1]** reported that gold nanoparticles biosynthesized using ***A. deliciosa*** extracts were capable of triggering apoptosis in HepG2 cells, suggesting a nanoparticle-based delivery strategy for enhancing therapeutic precision.Finally, a recent 2023 report [19**]** demonstrated that a phenolic-rich plant extract could induce caspase-dependent apoptosis in HepG2 cells, in line with the observations made in the current study. This emphasizes the role of phenolic compounds as core therapeutic candidates in liver cancer management.

6. Nutritional and Functional Relevance

A broad nutritional review [15] classified *Actinidia* fruits as functional foods, linking their antioxidant load with liver cancer prevention, immune modulation, and metabolic regulation.

3. MATERIALS AND METHODS

3.1 Extraction and Isolation of the Bioactive Compound

Fresh ripe fruits of *Actinidia deliciosa* were washed, peeled, and their pulp was homogenized using a mechanical blender. The homogenate was subjected to maceration using methanol (1:3 w/v) for 72 hours with intermittent shaking at room temperature. The methanolic extract was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C.The resulting crude extract was subjected to silica gel column chromatography using a gradient elution of solvents with increasing polarity (e.g., hexane, chloroform, ethyl acetate, methanol). Fractions were collected and tested for bioactivity. The most active fraction, as determined by preliminary cytotoxic screening, was further purified and structurally characterized using:

- GC-MS (Gas Chromatography-Mass Spectrometry): To identify volatile bioactive components.
- FT-IR (Fourier Transform Infrared Spectroscopy): To detect functional groups (e.g., –OH, C=O, C–O–C).
- NMR (Nuclear Magnetic Resonance Spectroscopy): ^1H and ^13C NMR were used to determine the compound's structure and confirm purity.

3.2 Cell Line and Culture Conditions

The HepG2 human hepatocellular carcinoma cell line was procured from a certified biorepository. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM**)** supplemented with:

- 10% fetal bovine serum (FBS)
- 1% penicillin-streptomycin (100 IU/mL and 100 µg/mL)
- 1% L-glutamine

3.3 Cytotoxicity Assay - MTT

The MTT assay (Mosmann, 1983) was used to assess the cytotoxic potential of the isolated compound.

Procedure:

- HepG2 cells were seeded at a density of 1 × 10^4 cells/well in a 96-well plate and incubated for 24 hours.
- Cells were treated with various concentrations of the purified compound (2, 4, 6, 8, and 10 µg/mL) for 24 and 48 hours.

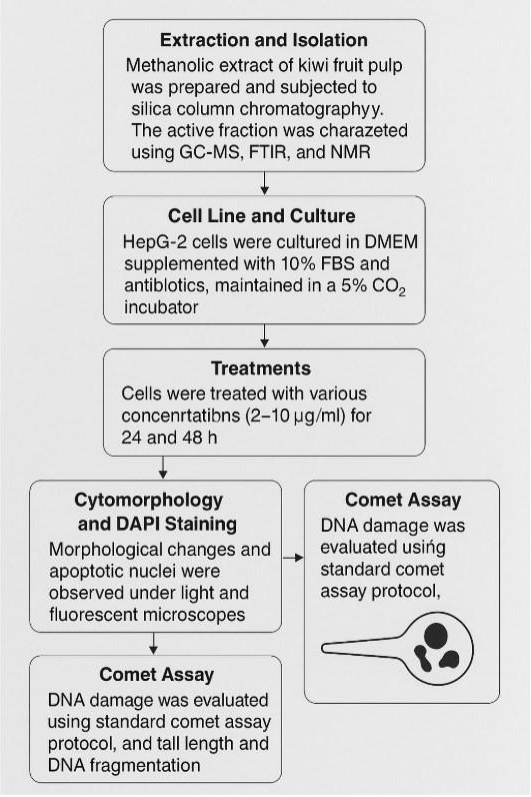

Figure no 2: METHODOLOGY

3.4 Cytomorphological Assessment and DAPI Staining

Light Microscopy:

- Morphological changes such as cell rounding, shrinkage, detachment, and membrane blebbing were observed in treated HepG2 cells compared to untreated controls.

DAPI Staining (4′,6-diamidino-2-phenylindole):

- After treatment, cells were fixed in 4% paraformaldehyde and stained with DAPI (1 µg/mL) for 10 minutes.
- Stained nuclei were observed under a fluorescence microscope.
- Apoptotic cells exhibited nuclear condensation, fragmentation, and chromatin margination.

3.5 Comet Assay (Single-Cell Gel Electrophoresis)

The comet assay was performed to detect DNA damage at the single-cell level:

Steps:

- Treated and control HepG2 cells were embedded in 0.5% low-melting agarose on pre-coated microscope slides.
- Cells were lysed in cold alkaline lysis buffer (pH >13) for 1 hour at 4°C.
- Electrophoresis was conducted at 25 V and 300 mA for 20 minutes.
- Slides were neutralized and stained with ethidium bromide (2 µg/mL).
- DNA migration patterns were observed under a fluorescence microscope.

Parameters analyzed:

- After incubation, 20 µL of MTT reagent (5 mg/mL) was added to each well and incubated for 4 hours at 37°C.
- The formazan crystals formed by mitochondrial dehydrogenases were dissolved using DMSO (dimethyl sulfoxide).
- Absorbance was measured at 570 nm using a microplate reader.

- Tail length
- % DNA in tail
- Olive tail moment

4. RESULTS AND DISCUSSION

- **Cytotoxic activity (MTT assay):**
- The isolated compound exhibited potent inhibitory effects on HepG-2 cell viability in a clear dose- and time-dependent manner. The calculated IC_{50} values were 12.84 µg/mL at 24 h and 3.90 µg/mL at 48 h, indicating enhanced efficacy with prolonged exposure.
- **Nuclear fragmentation (DAPI staining):**
- Fluorescence imaging following DAPI staining confirmed prominent nuclear fragmentation and chromatin condensation in treated groups—hallmarks of apoptosis
- **DNA damage (Comet assay):**
- Single-cell gel electrophoresis showed significant DNA strand breaks in treated cells, evidenced by increased comet tail length and higher percentage of tail DNA compared to controls, consistent with DNA fragmentation associated with apoptosis.
- **Structural characterization (GC-MS, FT-IR, and NMR):**
- Comprehensive chemical analyses—gas chromatography–mass spectrometry, Fourier-transform infrared spectroscopy, and both ^1H and ^{13}C NMR—validated the structure of the bioactive compound responsible for the observed biological effects.

Assay	Key Measurement	Value / Observation
Assay	Key Measurement	Value / Observation
MTT (24 h)	IC_{50}	12.84 µg/mL

MTT (48 h)	IC_{50}	3.90 µg/mL
Morphology / DAPI	Apoptosis indicators	Cell rounding, shrinkage, nuclear condensation; nuclear fragmentation confirmed by fluorescence microscopy
Comet assay	DNA damage (tail length / % DNA)	Significant increases vs. control; elevated comet tail and tail-DNA percentage
Structural (GC-MS, FT-IR, NMR)	Compound validation	Confirmed chemical identity of the bioactive agent

Table 1: Summary of Hep G-2

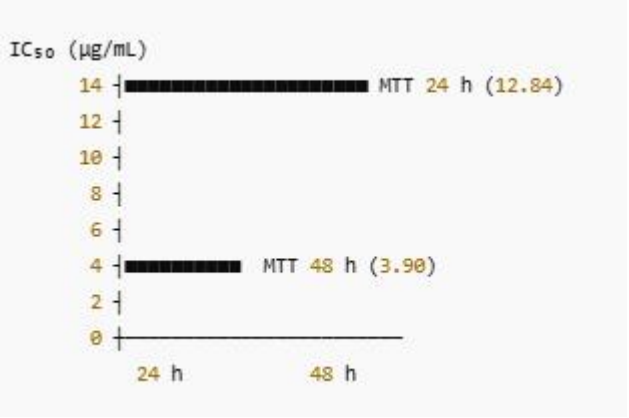

Figure no 3: Inhibitary Concentration with time

CONCLUSION

The isolated compound demonstrates strong cytotoxic activity against HepG2 cells, with IC_{50} values of 12.84 µg/mL at 24 h and 3.90 µg/mL at 48 h, confirming a clear dose- and time-dependent inhibitory effect. Complementary assays—including morphological analysis, DAPI staining, comet assay, and advanced spectroscopic techniques (GC-MS, FT-IR, NMR)—collectively validate its capacity to induce apoptosis and DNA damage. The consistent convergence of results from these diverse methodologies robustly supports the compound's pro-apoptotic and genotoxic activity in HepG2 cells.

This study demonstrates the potent anti-proliferative and pro-apoptotic effects of a phenolic compound isolated from *Actinidia deliciosa* against HepG-2 cells. The compound effectively induced DNA damage and apoptosis, indicating its promise as a natural therapeutic agent for liver cancer. Future work should include mechanistic studies involving apoptotic pathways, in vivo validations, and formulation development for clinical translation.

6. FUTURE WORK

- **Mechanistic Pathway Analysis:**Conduct Western blotting or RT-PCR to quantify expression changes in apoptosis-related proteins (e.g., Bax, Bcl-2, caspases) and confirm engagement of intrinsic and/or extrinsic pathways—similar to methodologies used in related compounds targeting PARP-1 and PI3K/Akt pathways .
- **Selectivity Profiling-**Test the compound on normal human liver cell lines (e.g., THLE-2, WI-38) to determine therapeutic selectivity and safety margin, as recommended in standard cytotoxic compound evaluation protocols
- **In Vivo Efficacy and Toxicity Studies**-Advance to animal models (e.g., HepG2 xenografts or chemically induced liver cancer in rodents) to assess in vivo antitumor efficacy, pharmacokinetics, and systemic toxicity profiles, following precedents set in phenoxyacetamide and coumarin derivative studies
- **Computational Docking and SAR Studies**-Utilize in silico molecular docking and dynamics to elucidate binding interactions between the compound and target proteins, refine structural analogs, and guide SAR optimization, mirroring successful efforts in natural product screening before biological assays.

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