Indian Journal of Biochemistry & Biophysics
Vol. 63, May 2026, pp. 557-573
DOI: 10.56042/ijbb.v63i5.24864

Combined therapeutic potential of kiwi leaf extract and Epigallocatechin gallate on A549 lung cancer cell line

Venkadeswaran Karuppasamy[1]*, Jyothi Kanagaraj[2]*, Venkatesh Srinivasan[3] & Sudha Chinnaiyan[4]

[1 & 3]Department of Biotechnology, P.S.R. Engineering College, Sevalpatti-626 140, Sivakasi, Tamil Nadu, India

[2]Department of Biotechnology, Kalasalingam Academy of Research and Education, Krishnankoil-626 126, Tamil Nadu, India

[4]PG and Research Department of Zoology, National College, Tiruchirappalli (PO)-620 001, Tamil Nadu, India

Received 15 October 2025; *revised 03 February 2026*

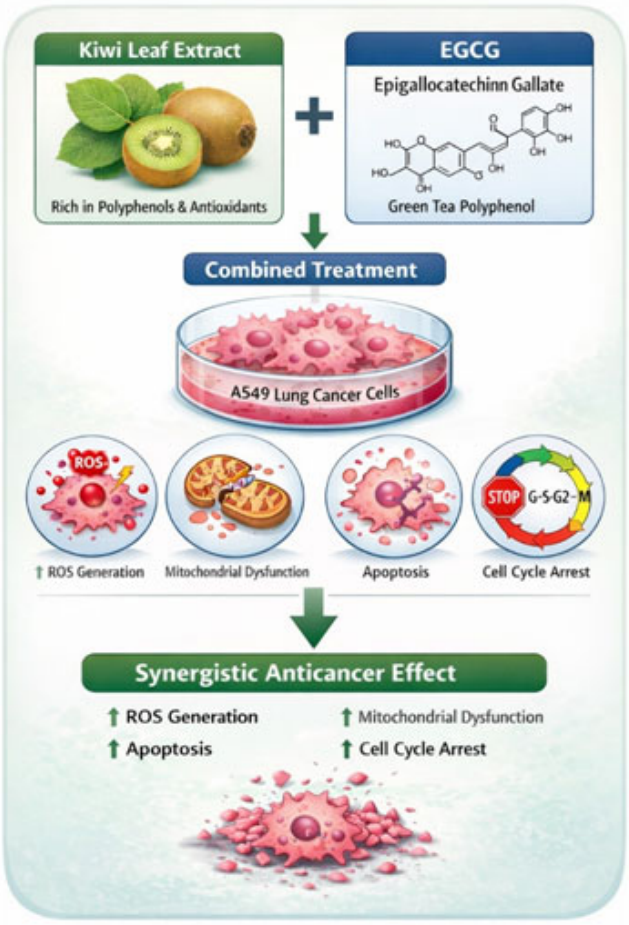
In this study, Epigallocatechin gallate (EGCG) was extracted using an aqueous extract from kiwi leaves (*Actinidia deliciosa*) to explore an alternative to traditional extraction methods such as citric acid and two-step extraction. The objective was to evaluate the combined therapeutic effects of kiwi leaf extract and EGCG on the A549 lung cancer cell line. Rich in bioactive compounds, kiwi leaves may enhance the anticancer efficacy of EGCG, a polyphenol found in green tea, strawberries, and other plants, known for its ability to inhibit cancer cell proliferation. Cell-based assays were employed to assess cell viability, apoptosis, and migration inhibition, while molecular analysis focused on signaling pathways involved in cancer progression. The findings highlight the potential of natural plant-based compounds, particularly kiwi leaf extract, in enhancing the therapeutic impact of EGCG. This combination may offer a promising strategy for lung cancer treatment by improving drug effectiveness and reducing side effects. Overall, this study provides innovative insight into the synergistic potential of kiwi leaf extract and EGCG as a natural and effective approach to lung cancer therapy.

Keywords: A549 cell line, Apoptosis, Kiwifruit leaf extract, Lung cancer

According to Noronha *et al.*[1,2] lung cancer is the most prevalent cause of cancer mortality in the population. According to current statistics published by the World Health Organization, lung cancer is the leading cause of death worldwide, with approximately 1.8 million fatalities attributable to this disease each year[6]. According to Nath *et al.*[4] among which, about 85% of lung cancer is non-small cell lung cancer. Most patients with advanced stage non-small cell lung cancer (NSCLC) have a limited life expectancy, and the lung cancer five-year relative survival rate is only approximately 15%, even with potentially curable options including surgery, chemotherapy, targeted therapies, and immunotherapy[5,6.] It highlights the urgent need for plant-based drugs that can potentiate therapeutic effect with minimal adverse effects. Plants, either raw or derivates have been the ancient Ayurveda medicine and have been used to treat multiple diseases. It is a source of many bioactive compounds separated from plant architectures for alternative therapy of diseases. Among them, polyphenols (PPs) have become a focus of interest due to their activities with various biological effects, including antioxidant, anti-inflammatory, and anticancer effects[7,8].

As reported by Hussain *et al.*[9] *A. deliciosa*, often known as the Kiwifruit, has strong nutritional advantages. Its leaves are also abundant in polyphenolic chemicals[10]. Recent research has shown that kiwi leaf extract has strong anti-inflammatory and antioxidant qualities, which may help explain its ability to fight cancer[11]. In 1972, a 58-year-old male Caucasian patient provided the A549 lung cancer cell line. Because of similar characteristics, these kinds of cells are frequently employed as models for research on non-NSCLC and human lung adenocarcinoma[12]. According to Hoffmann *et al.*[13] this cell line is particularly helpful for studying tumour biology, specifically cancer development and treatment resistance, as well as for determining the potential toxicity of a test material on cells. The researchers noted that A549 cells are utilized in the evaluation of novel lung cancer chemicals, the labelled cytotoxicity effects of chemotherapeutic drugs, and the impacts of

Correspondence:
Phone: +91-9500916826 (VK-Mob),
+91-9025303683 (JK-Mob)
E-mail: venkimalli09@gmail.com,
venkadeswaran@psr.edu.in (VK);
drjyothisamy@gmail.com, jyothi.k@klu.ac.in (JK)



Graphical abstract

radiation treatment[14]. The anticancer potential of EGCG, one of the main triphenolic-3-ols present in kiwis, has been studied. In cancer cell lines, EGCG has been shown in earlier studies to reduce angiogenesis, induce apoptosis, and depress cell viability[15,16]. Other beneficial bioactive include dietary fiber and carotenoids, which work in concert with EGCG to provide health-related benefits[17]. Additionally, according to Nagle *et al*.[18], it has anti-inflammatory properties, promotes weight reduction, and lowers the prevalence of chronic illnesses including cardiovascular disorders. Similarly, the solvent-based extraction has a more yield concentration, it is a viable approach for extracting EGCG from the extraction of kiwi leaves, despite the drawbacks of supercritical water extraction, ultrasound-assisted extraction, microwave-assisted extraction, and traditional methods[19,20]. According to Fujioka *et al*.[21], this technique is frequently associated with a homogenizer, which has been shown to significantly improve extraction efficiency and reduce processing time while preserving the integrity of the extracted molecule.

The chemical substance affects the biological processes like P13K/Akt and mitogen-activated protein kinase, signalling pathways both of which are crucial for cancer cell survival and proliferation[22,23]. Moreover, the EGCG, also can aid in the treatment of cancer by elevating the susceptibility of cancer cells to the effects of traditional chemotherapy drugs[24]. Kiwi leaf extract and EGCG both have anticancer effects *in vitro*, therefore their synergistic interaction in lung cancer cells is an intriguing study topic. In addition to minimizing the quantity of each chemical needed for effectiveness and potential side effects, the combined actions of natural compounds may lead to better treatment outcomes[25]. Collectively, these types of PPs are reported to be more efficient that when utilized individually at inhibiting the formation of malignancies[26]. Regarding this, researchers are interested in finding out more about how well EGCG and kiwi leaf extract work together on the A549 lung

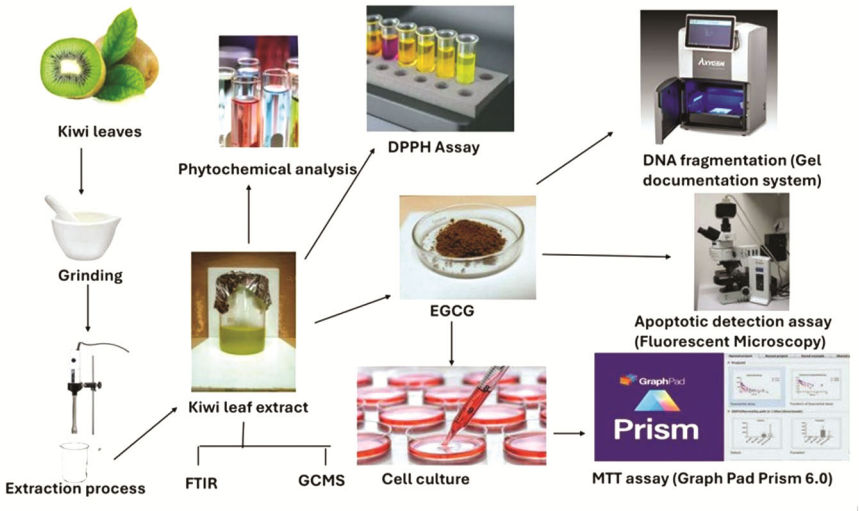


Fig. 1 — Experimental design illustrating EGCG extraction, treatment of A549 lung cancer cells, and subsequent analysis through different *in vitro* assays

cancer cell line[27]. This study used a multifaceted strategy to assess how well plant-based medications protect against lung cancer in humans (Fig. 1).

Materials and Methods

Chemicals and materials

Acetone, 2,2 Diphenyl-1-Picrylhydrazyl (DPPH), Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) (5 mg/mL) were from Sigma, (USA), dulbecco's modified eagle medium (DMEM) medium, 1X phosphate-buffered saline (PBS), (India). 96 well tissue culture plate and wash beaker were from Tarson (India). Penicillin/Streptomycin antibiotic solution, Trypsin- ethylenediamin etetraacetic acid (EDTA) was purchased from Gibco (USA), The Alexa Fluor® 488 annexin V/Dead Cell Apoptosis Kit was purchased from Thermo Scientific (USA), Fluorescent Imaging System, (ZOE, Bio-Rad, USA). minimum essential medium, ethidium bromide, agarose, phenol (C_6H_5OH), chloroform ($CHCl_3$), isoamyl alcohol ($C_5H_{12}O$), sodium dodecyl sulfate, tris-hydrochloric acid (HCl), EDTA, bromophenol blue, glacial acetic acid (CH_2COOH) were from Merk, 1X PBS was from Himedia, (India). 6 well tissue culture plate and wash beaker were from Tarson (India).

Preparation of Crude Kiwi Leaf extract

The *A. deliciosa's* leaf was collected during the growing season, ideally in late spring or summer when the leaves were at their ripest. The collected leaves were washed with distilled water. Then it was placed under sunlight for 2-3 days until it was fully dried[10]. The leaves were transferred to a mixer blender and it was ground into fine powder and then it was allowed to pass through 0.25 mm mesh screen. The powdered kiwi leaf was kept at -20°C until for extraction. As per the methodology given by Lv *et al.*,[28] was followed for the extraction of EGCG from the kiwi leaf. For this, we have taken 300 mL of 70% (*v/v*) $(CH_3)_2CO$ solution to perform solvent extraction method. 10 gm of powdered kiwi leaf was mixed with the and was homogenized under various atmospheric pressure for 2-3 min while maintaining the temperature of 30°C. The crude solution was filtered using filter paper to get pure filtrate. This was stored at 4°C for further analysis.

Isolation of Epigallocatechin gallate (EGCG)

The concentrated crude extract was subjected to liquid partitioning to enrich the polyphenolic fraction. Briefly, the extract was suspended in distilled water and successively partitioned with n-hexane (to remove non-polar components) and ethyl acetate. The ethyl acetate fraction, enriched in catechins, was collected and evaporated to dryness under reduced pressure[24].

Purification by column chromatography

The dried ethyl acetate fraction was loaded onto a silica gel column chromatography system (60–120 mesh). Elution was carried out using a gradient solvent system of chloroform: methanol (95:5 to 70:30, *v/v*). Fractions were collected and monitored by Thin-layer chromatography (TLC) using silica gel

plates and an appropriate mobile phase. Spots were visualized under UV light (254 and 366 nm) and by spraying with ferric chloride reagent to detect phenolic compounds [24].

Final purification and characterization

The pooled fractions were further purified by reverse-phase high-performance liquid chromatography (RP-HPLC) using a C18 column. The mobile phase consisted of water containing 0.1% formic acid (solvent A) and acetonitrile (solvent B), with a gradient elution program at a flow rate of 1.0 mL/min. Detection was carried out at 280 nm. The retention time of the isolated compound was compared with that of a commercial EGCG standard.

Confirmation of identity and purity

The identity and purity of the isolated EGCG were confirmed by HPLC analysis, and only fractions with purity ≥95% were used for subsequent biological assays. The purified EGCG was lyophilized and stored at −20 °C until further use.

Qualitative analysis of Phytochemicals

Initial phytochemical screening was conducted by investigating the techniques provided by Banu *et al*.[29]

Test of Alkaloids (Mayer's Test)

Equal volume of extract and potassium iodide solution was taken and observed for colour change. The appearance of whitish-yellow or cream-cream-colored precipitate indicates it is presence.

Test for Terpenoids (Salkowski Test)

To the extract, 2 mL of $CHCl_3$ and a few drops of sulfuric acid were added. Formation of reddish-brown ring shows the positive result of terpenoids.

Test for Flavonoids (Alkaline Reagent Test)

To the extract, few drops of dilute ammonium and concentrated HCl were added together. The development of a yellow coloration indicates the presence of flavonoids.

Test for Steroids (Schultz Test)

To the extract, 2 mL of $CHCl_3$, 2 mL of CH_3COOH and 2 mL of concentrated sulphuric acid was added. Greenish color shows the result of steroids.

Test for Phenols (Lead Acetate Test)

To the extract, 1 mL of lead acetate solution was added. Resulting precipitate shows the presence of phenols.

Test for Tannins (Lead Acetate Test)

To the extract lead acetate solution was added and observed for the result. The appearance of white precipitate indicates the presence of tannins.

Test for Amino acids (Ninhydrin Test)

To the extract, few drops of Ninhydrin solution was added and allowed this test tube to stand in water bath for 10 min. The emergence of purple or blue colour infers the presence of amino acids.

Test for Reducing sugars (Fehling's Test)

To the extract, equal volumes of Fehling's solutions A and B were added and heated. Formation of a brick-red precipitate shows the presence of reducing sugars.

In vitro Antioxidant activity

DPPH free radical scavenging assay

According to Sivaraj *et al*.[30] a DPPH free radical test technique was employed to evaluate the radical scavenging ability of the *A. deliciosa* aqueous fruit extract of leaves. 1 mL of a 0.1 mM DPPH solution in methanol was added with 1 mL of an aliquot of the aqueous extract at different concentrations. After that, it was left to stand for 30 min in the dark. 1 mL of methanol and 1 mL of the DPPH solution were combined to provide a control solution for reference. The vitamin C antioxidant has been employed as the standard of reference to assess the drop in absorbance at 517 nm.

$$\text{Scavenging effect (Inhibition \%)} = A_c - A_s / Ac \times 100$$

where,

A_c – Absorbance of control

A_s – Absorbance of sample

Characterization Studies

Fourier transform infrared spectroscopy (FTIR)

FTIR is the analytical tool that plays a key role in determining the functional groups of many organic and inorganic compounds. To detect solid sample components requires a spacer slide that limits the optical path length and sample volume. To clamp the spacer slice potassium bromide salt was used. The spectrum range was set at 500 to 4000 cm^{-1}, [31].

Gas-chromatography mass spectrometry (GC-MS)

Chromatographic column was HP-5MS with an elastic quartz capillary column. Helium was employed as the carrier gas, with a velocity of 1 mL min^{-1}. The GC procedure began at 50°C, then increased to 250°C at 8°C min^{-1} rate, and finally to 300°C at a rate of 5°C min^{-1}. The range of MS program scanning quality was 300-600 amu. The ionization current was 150 mA, and ionization voltage was 70eV.

Ion source and quadrupole temperature were 230°C and 150°C, respectively[12].

Experimental methods

Cell culture and maintenance

Human alveolar basal epithelial cell line A549 was purchased at NCCS, Pune, along with cultured in DMEM enriched with 100 µg/mL streptomycin, 100 µg/mL penicillin, and 10% FBS. Within the ambient environment, the culture was maintained at 37°C with 5% CO_2. Cells were subcultured at 70–80% confluency using 0.25% trypsin EDTA. Only cells within a defined passage range were used for all experiments. Prior to treatment, cells were allowed to adhere overnight and were then treated with kiwi leaf extract, purified EGCG, or their combination at the indicated concentrations and incubation periods. Untreated cells served as control.

MTT Assay-Based Evaluation of Kiwi EGCG Cytotoxicity in A549 Cells

The test sample Kiwi. Epigallocatechin gallate (K. EGCG) was tested for *in vitro* cytotoxicity, using A549 cells by MTT assay. Briefly the cultured A549 cells were harvested by trypsinization and pooled in a 15 mL tube. Then, the cells were plated at a density of 1×105 cells/mL cells/well (200 µL) into the 96-well tissue culture plate in DMEM medium containing 10% FBS and 1% antibiotic solution for 24-48 h at 37°C. Each of the wells were treated with different concentrations of the test sample in a serum-free DMEM medium after being cleaned with sterile PBS. A total of three copies of each sample were made, and those cells were cultured for 24 h at 37°C in a humidified 5% CO_2 incubator. After incubation, MTT (10 µL of 5 mg/mL) was added to each well and the cells were incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) was aspirated off the wells and washed with 1X PBS (200 µL). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using microplate reader (Thermo Fisher Scientific, USA). The absorbance correlates with the number of viable cells, as more formazan indicates greater metabolic activity (Andrea Salis *et al*., 2021). The percentage cell viability and IC_{50} value were calculated using Graph Pad Prism 6.0 software (USA)[28].

$$\text{Cell Viability \%} = \text{Test}_{OD} / \text{Control}_{OD} \times 100$$

Assessment of Kiwi EGCG-Induced Apoptosis in A549 Cells

Briefly, 5×10^5 cells/mL of A549 were seeded into the 96 well tissue culture plate in a DMEM medium containing 10% FBS and 1% antibiotic solution for 24-48 h at 37°C. The wells were washed with sterile PBS and treated with 44.41 µg/mL of Kiwi. Epigallocatechin gallate (K. EGCG) test sample in a serum-free DMEM medium. The plate was incubated at 37°C in a 5% CO_2 incubator for 24 h. After incubation, 10 µL of Alexa Fluor and 10 µL of PI were added to the wells and mixed gently and incubated for 15 min. After incubation 400 µL of 1x Annexin binding buffer was added and mixed gently. Finally, the plate was centrifuged at 800 rpm for 2 min and evaluated immediately within an h, and the cells were examined by a fluorescence imaging system (ZOE, Bio-Rad, USA)[32].

DNA fragmentation assay

Briefly, A549 cells were seeded in a six-well plate at a density of 1×10^6 cells/well and incubated for 24 h at 37°C in a humidified 5% CO_2 incubator. The wells were washed with sterile PBS and treated with Kiwi. Epigallocatechin gallate (K. EGCG) sample at the concentration of 44.41 µg/mL in a serum free DMEM medium and incubated for 24 h at 37°C in a humidified 5% CO_2 incubator. The cells were harvested by trypsinization in a 1.5 mL tube and centrifuged at 10,000 rpm for 10 min. After centrifugation the supernatant was discarded and 500 µL of lysis buffer was added to the cell pellet, incubated in room temperature for 1 h. Then 700 µL of C_6H_5OH- $CHCl_3$ - $C_5H_{12}O$ was added and mixed by inversion, and then centrifuged at 10,000 rpm for 5 min. The Aquatic phase was transferred into a new Eppendorf tube. An equal volume of cold isopropyl was added into tubes, and mixed gently by inversion. The tubes were then centrifuged at 10,000 rpm for 5 min and discarded the supernatant followed by the pellet was air-dried for 30 min. Then, the dried DNA was dissolved in 50 µL distilled water. Furthermore, the extracted DNA was quantified by UV spectrophotometer using optical density at 260 nm and 280 nm. The OD value of 1.83 indicated that the quality of DNA was found to be good without protein/RNA contamination. Then the equal amount of each DNA samples was run at 0.8% of agarose gel electrophoresis along with 100 bp ladder. The image was captured by gel documentation system[33.]

Analysis of Caspase -3, Bcl-2 and Bax Protein Expression by Immuno blot

A549 human lung adenocarcinoma cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin under standard conditions (37 °C, 5% CO_2). Cells were seeded in 6-well plates and allowed to attach overnight. Subsequently, cells were treated with crude kiwi leaf extract, purified EGCG, or a combination of kiwi leaf extract and EGCG at the indicated concentrations for 24 h. Untreated cells served as the negative control. Following treatment, cells were washed twice with cold phosphate-buffered saline (PBS) and lysed using ice-cold RIPA buffer supplemented with protease inhibitor cocktail. Cell lysates were incubated on ice for 30 min with intermittent vortexing and then centrifuged at 12,000 × g for 15 min at 4 °C to remove cellular debris. The supernatants containing total cellular proteins were collected, and protein concentrations were determined using the Bradford assay. Proteins, separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), were subsequently electrophoresis transferred onto a polyvinylidene fluoride membrane using a semi-dry blotting apparatus (Bio-Rad, Hercules, California, USA) at 24 V for 1 h. Staining with Ponceau S solution was performed to confirm the successful transfer of proteins. To block non-specific protein binding, the membranes were incubated with 5% non-fat dry milk in Tris-buffered saline (pH 7.5) containing 0.1 % (*v/v*) Tween 20 for 2 h. The membranes were then incubated overnight at 4 °C with primary antibodies against Bcl-2 and Bax, diluted according to the manufacturer's instructions. β-actin was used as an internal loading control. Specific antibody against Caspase-3 (dilution 1:1000; Sigma, St. Louis, Missouri, USA), and β-actin (dilution 1:3000; Sigma, St. Louis, Missouri, USA), Bcl-2 (dilution 1:200; Santa-Cruz, California, USA), Bax (dilution 1:200; Santa-Cruz, California, USA) were applied to the blots. Immuno-reactivity was visualized using alkaline phosphatase conjugated to anti-mouse IgG secondary antibodies and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium chloride (Genei, Bengaluru, India). To quantify even subtle differences in band intensities, densitometry analysis was performed on scanned images of the membranes. Quantity One software (Bio-Rad, Hercules, California, USA) was used for the analysis of these scanned images.

Statistical Analysis

All experiments, including MTT cytotoxicity assays, caspase-3 activity assays, and Western blot analyses of Bcl-2 and Bax, were performed in triplicate and repeated independently at least three times. Data are presented as mean ± standard deviation (SD). Statistical analyses were carried out using Statistical Package for Social Sciences software package for Windows (version 16.0; IBM Corp., Armonk, NY, USA) Differences among multiple treatment groups (control, kiwi leaf extract, EGCG, and their combination) were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. For comparisons between two groups, Student's *t*-test was applied where appropriate. A p-value of < 0.05 was considered statistically significant. For Western blot analysis, band intensities were quantified by densitometry, normalized to β-actin, and expressed as fold change relative to the control group prior to statistical evaluation.

Results and Discussion

Effect of Kiwi leaf extract, Purified EGCG, and their combination on A549 cell viability

The effects of crude kiwi leaf extract, purified EGCG, and their combination on A549 cell viability were evaluated using the [MTT] assay. Cells treated with either crude kiwi leaf extract alone or purified EGCG alone exhibited a dose-dependent reduction in cell viability compared to the vehicle control ($P< 0.05$), indicating that both treatments possess inherent cytotoxic activity. Notably, treatment with the combination of crude kiwi leaf extract and purified EGCG resulted in a significantly greater reduction in cell viability than either the extract alone or EGCG alone at equivalent concentrations ($P< 0.01$). At the tested concentration, the combination treatment reduced cell viability to 41.6%, compared to 72.4% and 68.9% for kiwi leaf extract alone and purified EGCG alone, respectively. These results demonstrate that while both individual treatments are biologically active, their combined administration enhances the overall anticancer effect, suggesting a beneficial interaction between constituents present in the crude extract and purified EGCG (Fig. 2).

Qualitative analysis of Phytochemicals

It is evident from the existence of many categories of bioactive chemicals that plants can have therapeutic effects on human physiological systems. Among the several bioactive substances, alkaloids and flavonoids are particularly well-known for their antibacterial, anti-

inflammatory, and antioxidant properties, which enable them to have a significant influence on both conventional and alternative medicine[2]. The results of a thorough phytochemical examination of kiwi leaf extract showed the presence of components that support personal health, including alkaloids, flavonoids, steroids, tannins, and amino acids (Table 1). The significant antioxidant activity of flavonoids, that protects cells from oxidative stress, is commonly paired with the strong analgesic and antispasmodic properties of alkaloids. It additionally indicates positive outcomes for steroids and tannins, which may help with anti-inflammatory responses and immunological control. Nevertheless, the study also implies the absence of additional compounds such terpenoids, saponins, and reducing sugars. According to Al-Kawaz *et al.*[34], this indicates that the phytochemical status of kiwi leaves may differ from that of other therapeutic plants that often contain these chemicals. In addition to having potential uses in herbal medicine, this unique configuration of bioactive chemicals may initiate certain physiological consequences (Fig. 3).

DPPH Free radical scavenging assay

The antioxidant activity of kiwi leaf extract, as determined by the DPPH test, is displayed in the table below. It is among the most used techniques for identifying decreasing free radicals in a sample. The purple product known as the DPPH salt is converted to a yellow-colored compound when it comes into touch with hydrogen donar (Table 2). The resulting color change indicates that the substance being extracted can scavenge free radicals. The kiwi leaf extract's IC_{50} value, indicating the concentration required to inhibit 50% of the DPPH radicals, ranged from 1.14 to 0.92 µg/mL. Findings show that the kiwi leaf has an elevated level of free radical scavenging activity, which is a potential capacity for neutralizing free radicals. These findings are consistent with the research findings of Salama Zeinab *et al.*[35]. Furthermore, kiwi leaf's antioxidant properties could be employed in a variety of ways, including enhancing one's health and reducing oxidative stress through food products (Fig. 4).

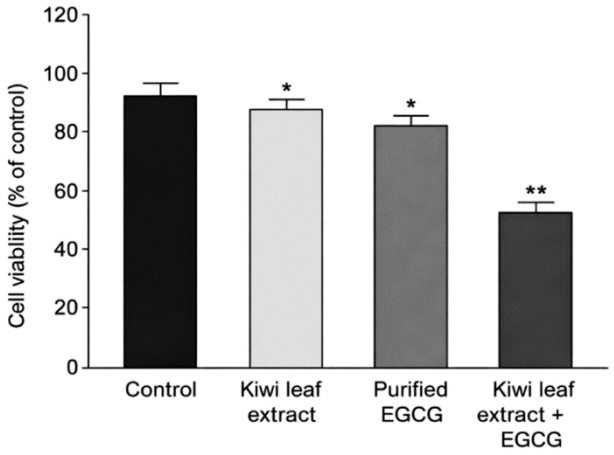


Fig. 2 — Comparative effects of kiwi leaf extract, purified epigallocatechin gallate (EGCG), and their combination on cell viability

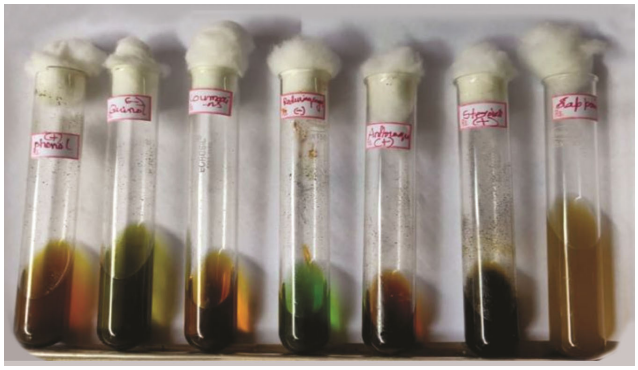


Fig. 3 — Phytochemical tests performed for the identification of bioactive compounds present in the kiwi leaf extract

Table 1 — Qualitative analysis of phytochemicals in kiwi leaf extract, highlighting the presence of various bioactive compounds.

Phytochemical constituents	$(CH_3)_2CO$ Extract	Colour
Alkaloids	+	Cream coloured precipitate
Flavanoids	+	Yellow
Steroids	+	Green
Tannins	+	White
Aminoacids	+	Blue
Terpenoids	-	-
Reducing sugars	-	-
Saponins	-	-

Table 2 — DPPH assay results demonstrating the antioxidant activity of the kiwi leaf extract based on its radical scavenging ability

Concentration	A_s	A_c	%RSA	IC_{50}
1	0.776	0.9	3.777	0.924
2	0.638	0.9	19.11	0.979
3	0.465	0.9	38.33	1.034
4	0.294	0.9	57.33	1.088
5	0.126	0.9	76	1.143

Fourier Transform Infrared Spectroscopy (FTIR)

The table represented below shows the FTIR analysis of Kiwi leaf extract which was attained by using solvent extraction method. This result in obtaining the peak value which corresponds to its particular wavelength that reveals various functional groups present in the sample. Preferably, powerful and wide-ranging O-H stretching absorption peaks was noticed between 3575-3205 cm^{-1}, infers the presence of hydroxyl radical, which are rapidly interact with alcohols and phenolic compounds (Table 3). In next to that, between the range of 1460-1400 cm^{-1} reveals CH_2 & CH_3 bending, indicating the appearance of alkanes in the sample. A peak at 3000-2840 cm^{-1} infers the C-H stretching, whereas in 1090-1020 cm^{-1} reflects both C-O stretching and O-H distortion, which are the attributes of alcohols, ethers, carboxylic acid and esters. The presence of these functional groups marks the complex behaviour of the chemical components within the kiwi leaf extract, posing a mixture of hydrophilic and hydrophobic components. In addition to that, a clear peak at 1650-1640 cm^{-1} reflects to C=C stretching, which belongs to the family of alkenes. Peak at 881 cm^{-1} shows an anomeric carbon frequency, evolved because of the $(CH_3)_2$ CO employed during the extraction process. Together these findings showcase the initial chemical constituents of the kiwi leaf extract includes alkenes, alkanes, alcohols, ethers, carboxylic acid and esters, which correlates with the report by Dong *et al*[36]. These compounds deliver to the bioactivity of kiwi leaf extract and also plays a vital role in medicine (Fig. 5).

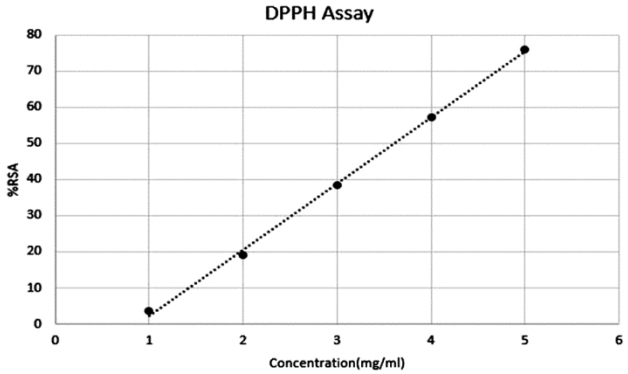


Fig. 4 — A plot of concentration vs. %RSA (Radical Scavenging Activity) for Kiwi extract, illustrating the antioxidant potential at different concentrations

High-performance liquid chromatography (HPLC) Analysis of Purified EGCG

High-performance liquid chromatography (HPLC) was employed to confirm the identity and purity of EGCG isolated from the kiwi leaf extract. The chromatographic profile of the purified fraction showed a single, sharp, and symmetrical peak with a retention time identical to that of the commercial EGCG standard analysed under identical chromatographic conditions. The absence of additional significant peaks indicated effective removal of co-eluting polyphenols and other extract constituents during the purification process. Quantitative analysis demonstrated that the isolated compound possessed a purity of ≥95%, confirming successful purification of EGCG from the crude kiwi leaf extract. Importantly, this purified EGCG was subsequently used as an independent test compound

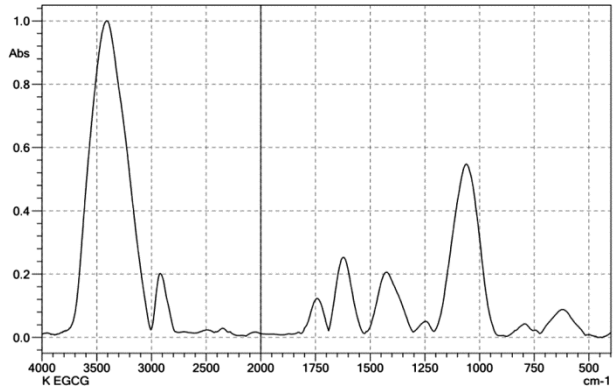


Fig. 5 — Graph illustrating the relationship between wavelength and absorbance, depicting the spectral characteristics of the kiwi leaf extract

Table 3 — FTIR detection results showing the identification of functional groups in the Kiwi leaf extract, based on its infrared absorption spectrum

Spectral range (cm^{-1})	Absorption peak (cm^{-1})	Absorption peak attribution	Chem composition
	Ethanol		
3575-3205	3417	O-H Stretching	Alkanes
3000-2840	2976	C-H Stretching	Alcohols, ethers
1650-1640	1650	C=C Stretching	Alkenes
1460-1400	1454	CH2, CH3	Alkanes
1090-1020	1090	C-O Stretching-H deformation	COOH, Ester
881	881	Anomeric carbon	Alkenes

in biological assays, while the crude kiwi leaf extract was evaluated separately. For combination studies, the purified EGCG was externally supplemented to the crude extract at defined concentrations, ensuring that the observed biological effects could not be attributed solely to the endogenous EGCG content of the extract (Fig. 6).

Representative reverse-phase HPLC chromatograms showing (A) commercial EGCG standard and (B) EGCG purified from kiwi leaf extract. Both chromatograms were recorded at 280 nm under identical chromatographic conditions. The purified fraction exhibited a single dominant peak with a retention time matching that of the EGCG standard, confirming the identity of the isolated compound. The absence of additional peaks in the purified sample indicates a purity of ≥95%.

Quantitative Estimation of EGCG by HPLC

Quantitative analysis of epigallocatechin gallate (EGCG) in kiwi leaf extract was performed using high-performance liquid chromatography (HPLC) with an external standard calibration method. Chromatographic separation was carried out on an HPLC system equipped with a quaternary pump, autosampler, column oven, and photodiode array (PDA) detector. Separation was achieved using a reverse-phase C18 column (250 × 4.6 mm, 5 µm particle size) maintained at 30°C. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile), delivered at a flow rate of 1.0 mL/min using a gradient elution program. The detection wavelength was set at 280 nm. The injection volume was 20 µL, and the total run time was [30 min]. A stock solution of authentic EGCG standard was prepared in methanol and serially diluted to obtain calibration standards over the concentration range of (5–100 µg/mL). The calibration curve was constructed by plotting peak area versus concentration, and linearity was confirmed with a correlation coefficient (R^2) greater than 0.99. Kiwi leaf extract samples were dissolved in methanol, filtered through a 0.22 µm syringe filter, and analysed under identical chromatographic conditions (Fig. 7). EGCG in the samples was identified by comparing retention time and UV spectra with those of the authentic standard and quantified using the external calibration curve. The EGCG content was expressed as milligrams of EGCG per gram of dry extract (mg/g).

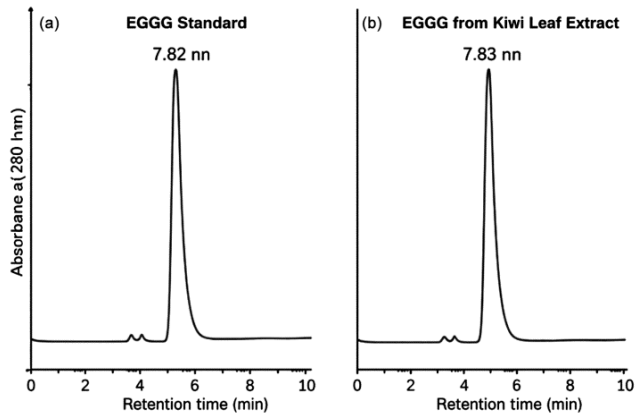


Fig. 6 — HPLC chromatograms of EGCG standard and EGCG isolated from kiwi leaf extract

Gas-Chromatography Mass Spectrometry (GC-MS)

The GC-MS analysis of Kiwi leaf extract was showed in the table given below. It spots a variety of bioactive compounds. Among the primary components identified were 3,5-bis(1,1-dimethylethyl),

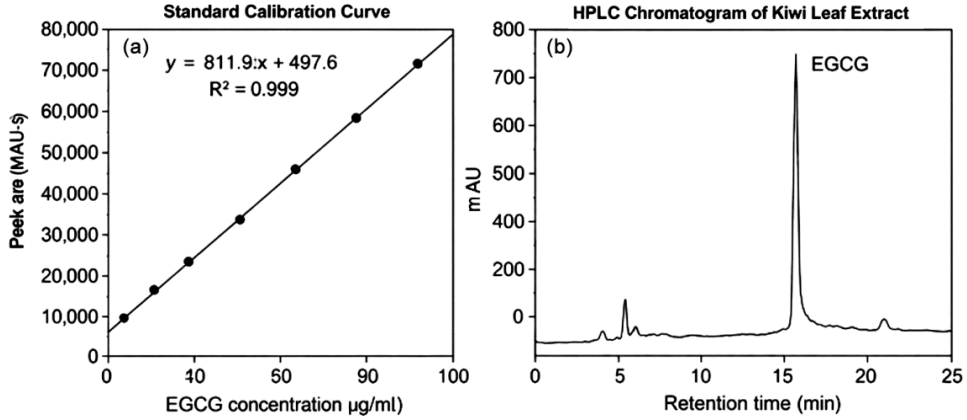


Fig. 7 — Quantification of EGCG in Kiwi leaf extract by HPLC. (a) Standard calibration curve of EGCG showing good linearity (R^2=0.999); and (b) HPLC Chromatogram of kiwi leaf extract with a prominent peak an identified as EGCG at a retention time of 12.5 min

Table 4 — GC-MS Analysis of Kiwi leaf extract

Peak	R. Time	Area	Area %	Height	Height %	Name
1	7.700	2459130584	90.96	102658463	50.50	3-Cyclopentyl-1-propyne
2	8.339	27317001	1.01	5706243	2.81	1-Hexanol,2-ethyl
3	11.620	37653440	1.39	13590170	6.69	Dodecane
4	12.342	2766198	0.10	1016441	0.50	Cyclohexane,hexyl-
5	12.715	4111319	0.15	1606750	0.79	Dodecane,2-methyl-
6	14.385	3134429	0.12	1054832	0.52	Tricosyl heptafluorobutyrate
7	14.713	11673895	0.43	5483950	2.70	1-Dodecanol
8	14.825	51487108	1.90	23026737	11.33	Tetradecane
9	15.569	3573547	0.13	1608314	0.79	Cyclohexane,octyl-
10	15.721	5213771	0.19	2378620	1.17	Tetradecane,2-methyl-
11	15.878	4491185	0.17	2037383	1.00	Dodecane,1-chloro-
12	16.440	6649104	0.25	2825892	1.39	Phenol,3,5,-bis(1,1-dimethylethyl)-
13	17.442	23175862	0.86	11765346	5.79	1-Pentadecane
14	18.316	8313958	0.31	3902729	1.92	2-Methyltetracosane
15	18.763	2549287	0.09	117355	0.55	Heptadecane
16	19.861	22539123	0.83	11426375	5.62	1-Heptadecene
17	20.641	4599594	0.17	1854217	0.91	Heneicosane
18	21.755	10430390	0.39	2982173	1.47	1,2-Benzenedicarboxylic acid,butyl 2-ethylhexyl ester
19	22.045	10427897	0.39	5722785	2.82	1-Nonadecene
20	24.041	4438124	0.16	1510427	0.74	1-Heptacosanol
		2703675816	100.00	203275202	100.00	

1-pentadecene, 2-methyltetracosane, heptadecane, 1-heptadecane, heneicosane, 1-2-benzenedicarboxylic acid (butyl 2-ethylhexyl ester), 1-nanodecene, 1-heptacosanol, 3-cyclopentyl-1-propyne, 1-hexanol (2-ethyl-), dodecane, tricosyl heptafluorobutyrate, 1-dodecanol, tetradecane, octyl-cyclohexane, 2-methyl-tetradecane, and 1-chloro-dodecane. In total, 20 chemical components were detected, collaborates the findings by Dong *et al*., 2019[12]. Particular notable compound in this profile is 2,6-di-tert-butylphenol, a phenolic compound characterized by its substantial peak due to tert-butyl groups (Table 4). This molecular structure increases its effectiveness as free radical scavenger; these groups are enclosed by phenolic hydroxyl group, making it to contribute hydrogen atoms to diminish the free radicals. 2,6-di-tert-butylphenol is a phenolic compound which will disrupt the oxidative stress process occurring within the cellular components like DNA and will able to protect them. Thus, this safeguard role makes it as an excellent compound for minimizing the impact of tumor commencement and succession (Fig. 8).

Dose-Dependent Cytotoxic Effects of Kiwi-Derived EGCG on A549 Lung Cancer Cells

The result shows that the investigated compound has a clear concentration-dependent lethal effect on cell viability, showing promise as an anti-proliferative agent. The control group shows the highest optical density, indicating maximum metabolic activity and cell survival. However, as the concentration of the sample was enhanced, there was significant decrease in the OD values and cell viability percentages as an indication that the compound reduces the rate of growth of cells[37]. The Half- minimum inhibitory concentration was calculated 44.41 µg/mL. This indicates even at the lower concentrations the drug is effective. The IC_{50} value attest to the validity of study, the confidence interval ranging from 33.86 to 57.51 µg/mL. Moreover, the cytotoxic response is also moderately steep and persistent at all concentrations, as is supported by the Hill slope of -0.6780, which reflecting the compound continuous interaction with the cellular targets. The high R-squared value of 0.9169 as describe in the result shows that the effect of the observed lethality is highly significant, and conveys the fact that changes in inhibitor concentration and cell viability are strongly associated. From these data it is possible to state that the compound under consideration possesses rather powerful anti-proliferative properties, and therefore it can be used in therapy[38,39]. The observed concentration-dependent cytotoxicity calls for further

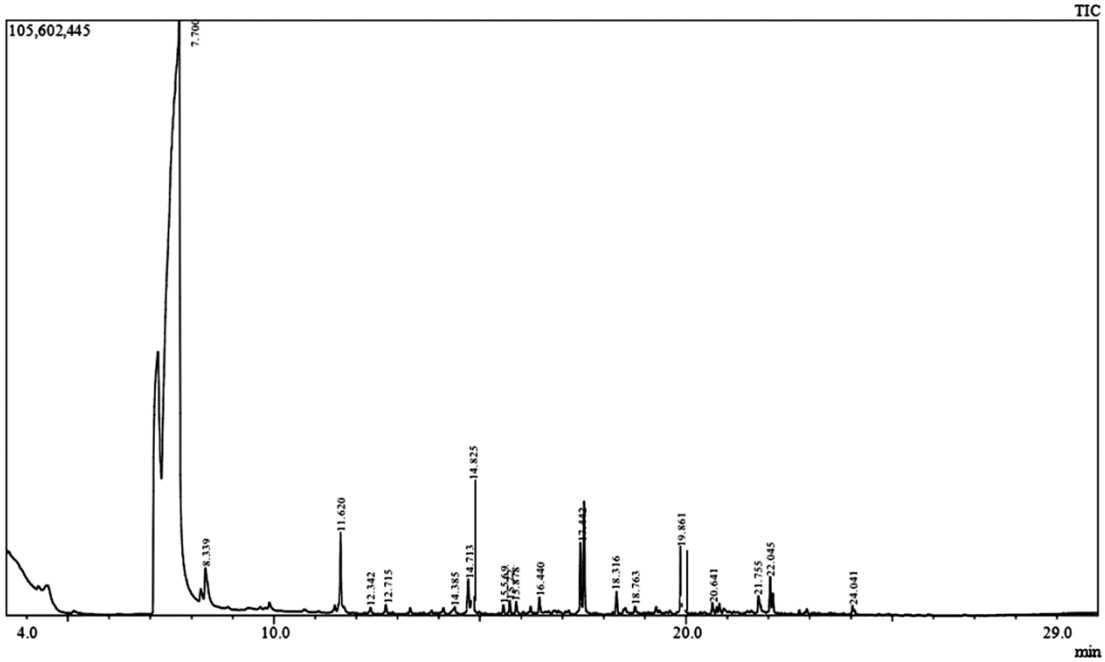


Fig. 8 — GC–MS total ion chromatogram (TIC) of kiwi leaf extract showing the distribution of phytochemical constituents based on retention time

exploration into the compound's mechanisms of action and anticancer potential. Thus, it establishes a good foundation particularly in cancer treatment and other disorders requiring aberrant cell proliferation[40].

The trends seen in the OD values are also supported by the cell viability percentages shown in the bar graph below. Cell viability in the control group is 100%, however it gradually declines as Kiwi. Epigallocatechin gallate (K. EGCG) concentrations rise. Cell vitality seems to be at its lowest at 500 µg/mL, indicating that Kiwi. Epigallocatechin gallate (K. EGCG) is useful in lowering cell viability. The visual data based on varying treatment concentrations applied to the cells and comparing them to the control is displayed in the image above. At a concentration of 500 µg/mL, the cells undergo distinct changes in terms of morphology. Some of them can look elongated, or have started to populate, which might indicate cytotoxicity. These results are in line with the objective of understanding the tumor-cracking capacity of biologically active compounds. These are suggestive outcomes that Kiwi. Epigallocatechin gallate (K. EGCG) could be a prospective anti-cancer agent since it affected the viability of the cancer cells inequitably in a concentration dependent manner (Fig. 9).

Cells were treated with increasing concentrations of Kiwi. Epigallocatechin gallate (K-EGCG) (0–500 µg/mL), and cell viability was assessed by MTT assay (Table 5). The reduction in cell viability is expressed as optical density (OD) measured at 570 nm. Control cells showed higher OD values compared to Kiwi. Epigallocatechin gallate (K-EGCG)–treated cells, indicating a concentration-dependent decrease in cell viability upon Kiwi (Table 6). Epigallocatechin gallate (K-EGCG) treatment. Data are represented as mean ± SD.

The figure illustrates the microscopic appearance of A549 cells exposed to different concentrations of the extract (1–500 µg/mL). The untreated control cells exhibit normal morphology, while treated groups show concentration-dependent alterations such as cell shrinkage, detachment, and reduced confluency, indicating cytotoxic effects (Fig. 10).

Fluorescence-Based Detection of Apoptosis Induced by Kiwi Epigallocatechin Gallate (K. EGCG) in Living Cells

The images below were captured using a fluorescence microscope following the treatment of living cells with Kiwi. Epigallocatechin gallate (K. EGCG). Untreated control cells show little to no fluorescein, indicating little to no positive apoptotic activity. In contrast, cells treated with 44.41 µg/mL of Kiwi. Epigallocatechin gallate (K. EGCG) have more areas of fluorescent signals; they produce both red and clear green fluorescence. This indicates that the cells in the control group are healthy and only a small

fraction of them die by apoptosis under normal conditions. Thus, orange fluorescence may represent early apoptotic cells, but green fluorescence may indicate late apoptotic or dead cells[41]. The presence of more apoptotic markers in the Kiwi. Epigallocatechin gallate (K. EGCG)-treated group than in the control group indicates that the therapy induces cell death[14]. The treated cells had higher fluorescence levels among the developing cells in contrast to the control cells (Fig. 11). The results suggest that Kiwi. Epigallocatechin gallate (K. EGCG) may induce apoptosis with an effective dosage of 44.41 µg/mL. The results obtained suggest that the concentration of Kiwi. Epigallocatechin gallate (K. EGCG) throughout the research may have demonstrated pro-apoptotic characteristics, partly because of its impact on cellular elements implicated in apoptosis[42].

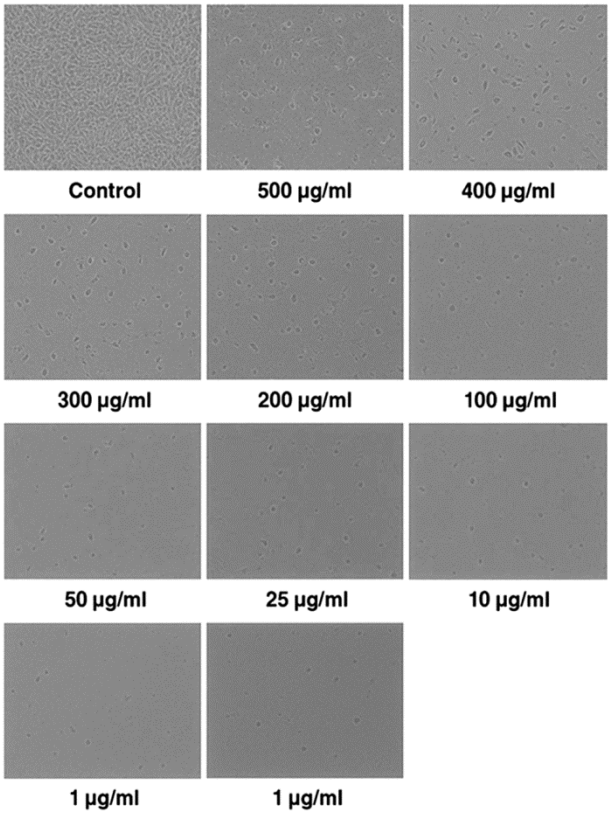


Fig. 10 — Morphological changes in A549 lung cancer cells treated with varying concentrations of kiwi leaf extract containing Epigallocatechin gallate (EGCG)

Activation of Caspase-3 in Response to Kiwi leaf extract, EGCG, and their combination

To further substantiate apoptosis induction at the molecular level, caspase-3 activation was evaluated in A549 cells following treatment with crude kiwi leaf extract, purified EGCG, and their combination. Western blot analysis revealed a marked increase in cleaved (active) caspase-3 levels in cells treated with the combination of kiwi leaf extract and EGCG compared to untreated controls[43]. Treatment with kiwi leaf extract alone or purified EGCG alone resulted in a moderate increase in cleaved caspase-3 expression. In contrast, the combination treatment produced a substantially higher level of caspase-3 cleavage, indicating enhanced activation of the apoptotic cascade. Densitometry analysis confirmed that cleaved caspase-3 expression in the combination group was significantly elevated compared to individual treatments ($P< 0.01$). These findings provide molecular confirmation that the observed reduction in cell viability and increased Annexin V/PI

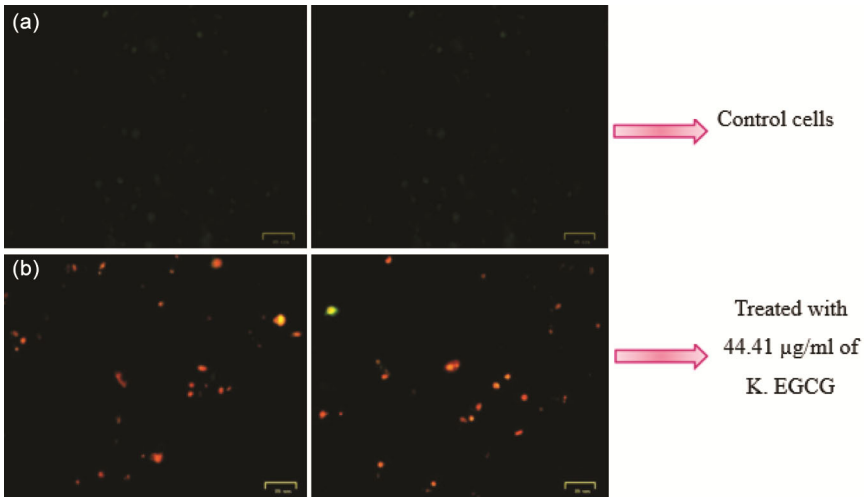


Fig. 11 — Microscopic images showing dead cells after treatment with the Kiwi. Epigallocatechin gallate (K. EGCG), highlighting cellular response and treatment efficacy

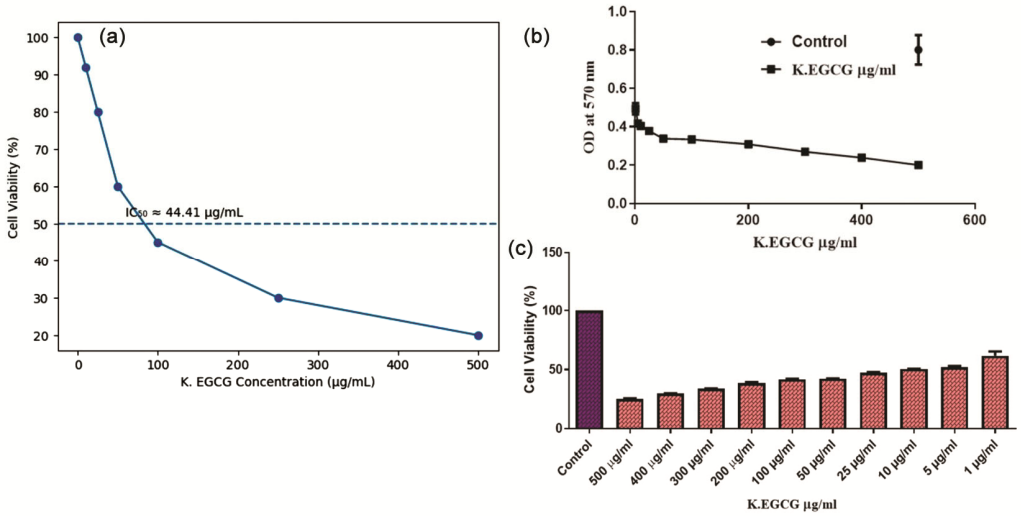


Fig. 9 — Effect of Kiwi. Epigallocatechin gallate (K-EGCG) on cell viability assessed by the MTT assay

Table 5 — MTT assay optical density measured at 570 nm

S. No.	Tested sample concentration (µg/ mL)	OD value at 570 nm (in triplicates)		
1	Control	0.877	0.723	0.8
2	500 µg/mL	0.197	0.204	0.200
3	400 µg/ mL	0.239	0.238	0.238
4	300 µg/ mL	0.272	0.267	0.269
5	200 µg/ mL	0.315	0.303	0.309
6	100 µg/ mL	0.331	0.337	0.334
7	50 µg/ mL	0.34	0.336	0.338
8	25 µg/ mL	0.383	0.373	0.378
9	10 µg/ mL	0.403	0.405	0.404
10	5 µg/ mL	0.411	0.423	0.417
11	1 µg/ mL	0.523	0.464	0.493

Table 6 — Effect of treatment on cell viability (%)

S. No.	Tested sample concentration (µg/ mL)	Cell viability (%) (in triplicates)			Mean Value (%)
1	Control	100	100	100	100
2	500 µg/ mL	24.625	25.5	25.062	25.062
3	400 µg/ mL	29.875	29.750	29.812	29.812
4	300 µg/ mL	34.00	33.375	33.687	33.687
5	200 µg/ mL	39.375	37.875	38.625	38.625
6	100 µg/ mL	41.375	42.125	41.75	41.750
7	50 µg/ mL	42.500	42.000	42.250	42.250
8	25 µg/ mL	47.875	46.625	47.25	47.250
9	10 µg/ mL	50.375	50.625	50.5	50.500
10	5 µg/ mL	51.375	52.875	52.125	52.125
11	1 µg/ mL	65.375	58	61.687	61.687

positivity are associated with caspase-dependent apoptosis (Fig. 12).

The pro-caspase-3 and cleaved caspase-3 in A549 cells treated with crude kiwi leaf extract alone, purified EGCG alone, or their combination for 24 h. β-actin was used as a loading control. The combination treatment markedly increased cleaved caspase-3 expression compared to individual treatments and control. Bar graph represents densitometric analysis of cleaved caspase-3

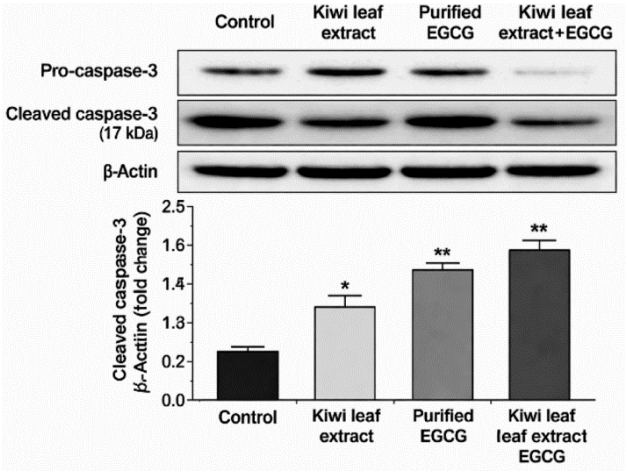


Fig. 12 — Activation of caspase-3 following treatment with kiwi leaf extract, purified EGCG, and their combination

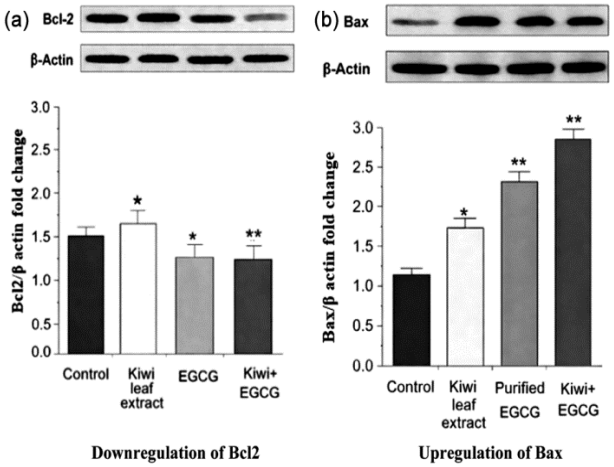


Fig. 13 — Modulation of Bcl-2 and Bax expression in A549 cells following treatment with kiwi leaf extract, purified EGCG, and their combination

normalized to β-actin. Data are expressed as mean ± SD of three independent experiments. $P< 0.05$ *vs* control; $P< 0.01$ vs extract alone and EGCG alone.

Activation of Bcl-2 and Bax in Response to Kiwi leaf extract, EGCG, and their combination

Treatment of A549 lung cancer cells with kiwi leaf extract, purified EGCG, and their combination resulted in marked alterations in the expression of key mitochondrial apoptotic regulators, Bcl-2 and Bax. Western blot analysis revealed that Bcl-2 (anti-apoptotic protein) expression was significantly downregulated, while Bax (pro-apoptotic protein) expression was correspondingly upregulated, particularly in the combination treatment group. Cells treated with kiwi leaf extract or purified EGCG alone showed moderate modulation of Bcl-2 and Bax expression compared to untreated controls, indicating partial activation of the intrinsic apoptotic pathway. Notably, the combined treatment produced a pronounced decrease in Bcl-2 levels accompanied by a substantial increase in Bax expression, leading to a significantly elevated Bax/Bcl-2 ratio. This shift is a critical determinant of mitochondrial outer membrane permeabilization and downstream caspase activation (Fig. 13).

The enhanced modulation of Bcl-2 and Bax observed with the combination treatment suggests a synergistic pro-apoptotic effect, rather than an additive response. While EGCG is known to induce apoptosis via mitochondrial pathways, the presence of additional bioactive phytochemicals in the crude kiwi leaf extract may potentiate EGCG-mediated signalling, resulting in amplified apoptotic responses. These findings corroborate the Annexin V/PI staining and caspase activation data, collectively demonstrating that the combined treatment promotes apoptosis through mitochondrial pathway regulation, thereby strengthening the mechanistic basis of the observed cytotoxic effects. Importantly, the inclusion of protein-level evidence addresses prior limitations and substantiates the claim that apoptosis induction is mediated, at least in part, through Bcl-2 family protein regulation[44].

The expression of the anti-apoptotic protein Bcl-2 and the pro-apoptotic protein Bax in A549 cells treated with crude kiwi leaf extract alone, purified EGCG alone, or their combination for 24 h. β-Actin was used as a loading control. Bar graphs represent densitometric analysis of Bcl-2 and Bax protein levels normalized to β-actin and expressed relative to untreated control. Data are presented as mean ± SD from three independent experiments. $P< 0.05$ *vs* control; $P< 0.01$ *vs* extract alone and EGCG alone.

Effect of Kiwi EGCG on DNA integrity

Although lane L is designated as blank, it contains a molecular weight marker called a DNA ladder. It was possible to determine the size of the bands from the ladder by comparing the distance moved by the bands of the DNA fragments in the other lanes (1 and 2). The control sample, lane 1, shows the usual unbroken band, indicating that there is no discernible DNA fragmentation on the control sample. No interference is anticipated during the DNA extraction process. In contrast, the DNA sample in Lane 2 was treated with 44.41 µg/mL of Kiwi. Epigallocatechin gallate (K. EGCG), resulting in a smeared band of

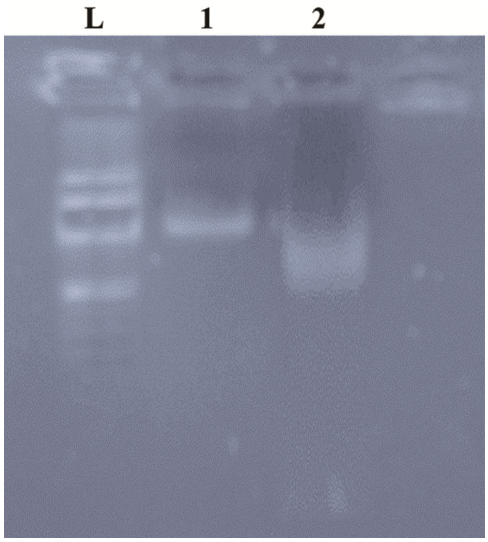


Fig. 14 — Effect of kiwi-derived epigallocatechin gallate (EGCG) on DNA integrity

DNA that points to DNA fragmentation. This implies that Kiwi. Epigallocatechin gallate (K. EGCG) may cause some kind of DNA damage or injury at this dose[45]. According to the smearing pattern in Lane 2, it is clear from the data above that Kiwi. Epigallocatechin gallate (K. EGCG) affects DNA integrity at the specified concentration[15]. According to Dilek Pandir[35], the smear could indicate breaks in DNA strands or other kinds of damage. However, the fact that the untreated DNA in the control lane band is still whole and undamaged after going through comparable experimental processes shows that the DNA is stable and undamaged. The value of Kiwi. Epigallocatechin gallate (K. EGCG) at 44.41 µg/mL may also be significant in studies on agents that damage DNA or the effects of Kiwi. Epigallocatechin gallate (K. EGCG) on cellular components, according to this paper (Fig. 14).

Agarose gel electrophoresis showing DNA fragmentation analysis. Lane L: DNA molecular weight marker (ladder); Lane 1: Control DNA showing intact band; Lane 2: DNA treated with 44.41 µg/mL of kiwi-derived EGCG showing smeared bands indicative of DNA fragmentation.

L. DNA Ladder
1. Control
2. Treated with 44.41 µg/mL of Kiwi. Epigallocatechin gallate (K. EGCG) sample

Conclusion

The present study demonstrates, at the *in vitro* level, that crude kiwi leaf extract, purified EGCG, and their combination exert cytotoxic effects in A549 lung cancer cells. The combination treatment showed enhanced activity compared with either treatment alone, as evidenced by reduced cell viability and increased apoptotic markers. Mechanistic insights derived from Annexin V/PI staining, caspase-3 activation, and modulation of Bcl-2 and Bax expression suggest the involvement of apoptosis-associated pathways in the observed cellular responses. Quantitative HPLC analysis confirmed the presence and purity of EGCG isolated from kiwi leaf extract, supporting its contribution to the biological effects observed. However, these findings are restricted to cell culture–based experiments and should be interpreted within the limitations of an *in vitro* model. Such systems do not fully recapitulate the complexity of tumor microenvironments, pharmacokinetics, bioavailability, or systemic toxicity encountered *in vivo*. Importantly, the study did not include normal lung epithelial cells or standard chemotherapeutic agents, and therefore conclusions regarding cancer selectivity, safety, or comparative efficacy cannot be drawn at this stage. Future investigations incorporating normal cell models, positive control drugs, and *in vivo* validation will be essential to further substantiate the biological relevance of these findings. Overall, this work provides preliminary *in vitro* evidence supporting the cellular effects of kiwi leaf extract and EGCG, either alone or in combination, and offers a foundation for more comprehensive mechanistic and translational studies.

Acknowledgement

We extend our sincere gratitude to Trichy Research Institute of Biotechnology Pvt. Ltd. (TRI Biotech) for their invaluable support and assistance in conducting cell line studies, apoptosis detection assays, and DNA fragmentation assays. Their expertise, technical guidance, and state-of-the-art facilities have greatly contributed to the success of our research. We deeply appreciate their collaboration and commitment to advancing scientific research.

Conflict of interests

All authors declare that no conflicts of interest.

References

1 Noronha V, Pinninti R, Patil VM, Joshi A & Prabhash K, 2016. Lung cancer in the Indian subcontinent. *South Asian J Cancer*, 5 (2016) 095.

2 Aggarwal V, Tuli HS, Tania M, Srivastava S, Ritzer EE, Pandey A, Aggarwal D, Barwal TS, Jain A, Kaur G. & Sak K, Molecular mechanisms of action of epigallocatechin

gallate in cancer: Recent trends and advancement. *Semin Cancer Biol*, 80 (2022) 256.
3 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA & Jemal A, 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin*, 68 (2018) 394.
4 Nath A, Sathishkumar K, Priyanka D, Sudarshan KL & Prashant M, A clinicoepidemiological profile of lung cancer in India-Results from National Cancer Registry Programme. *Indian J Med Res*, 155 (2022) 264.
5 Sivaraj C, Amshaja M, Saraswathi K & Arumugam P, Comparative study of antioxidant activities and GC-MS analysis of aqueous and ethanol fruit pulp extract of (green kiwi fruit) *Actinidia deliciosa* (A. Chev.) CF Liang & AR Ferguson. *J Drug Deliv Ther*, 9 (2019) 296.
6 Sunita D, Dinesh Kumar B, Suresh Kumar G, Evaluation of the apoptotic potential of a novel compound using DNA fragmentation assay. *J Appl Pharm Sci*, 3 (2013) 55.
7 Asker Mohsen S & Habiba A, A Active Constituents of Kiwi (*Actinidia Deliciosa Planch*) Peels and Their Biological Activities as Antioxidant, Antimicrobial and Anticancer. *Res. J Chem Environ*, 22 (2018) 52.
8 Pandir D, Protective effect of epigallocatechin-3-gallate on capsaicin-induced DNA damage and oxidative stress in human erythrocyes and leucocytes *in vitro*. *Cytotechnology*, 67 (2015) 367.
9 Hussain SZ, Naseer B, Qadri T, Fatima T & Bhat TA, Kiwifruit (*Actinidia deliciosa*) Morphology, Taxonomy, Composition and Health Benefits in Fruits Grown in Highland Regions of the Himalayas. *Springer International Publishing* (2021) 145.
10 Khiewkamrop P, Surangkul D, Srikummool M, Richert L, Pekthong D, Parhira S, Somran J & Srisawang P, Epigallocatechin gallate triggers apoptosis by suppressing de novo lipogenesis in colorectal carcinoma cells. *FEBS Open Bio*, 12 (2022) 937.
11 Du GJ, Zhang Z, Wen XD, Yu C, Calway T, Yuan CS. & Wang CZ, Epigallocatechin Gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. *Nutrients*, 4 (2012) 1679.
12 Ghasemi M, Turnbull T, Sebastian S & Kempson I, The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. *Int J Mol Sci*, 22 (2021) 12827.
13 Hoffmann M, et al., SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, 181 (2020) 271.
14 Riss TL, OBrien MA, Moravec RA, Kupcho K & Niles AL, Apoptosis marker assays for HTS. National Center for Advancing Translational Sciences, (2020).
15 Athirojthanakij W & Rashidinejad A, optimizing catechin extraction from green tea waste: Comparative analysis of hot water, ultrasound assisted, and ethanol methods for enhanced antioxidant recovery. *Food Sci Nutr*, 12 (2024) 5121.
16 Lv JM, Gouda M, Zhu YY, Ye XQ & Chen JC, Ultrasound-assisted extraction optimization of proanthocyanidins from kiwi (Actinidia chinensis) leaves and evaluation of its antioxidant activity. *Antioxidants*, 10 (2021) 1317.
17 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA & Jemal A, 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin*, 68 (2018) 394.
18 Nagle DG, Ferreira D & Zhou YD, Epigallocatechin-3-gallate (EGCG): chemical and biomedical perspectives. *Phytochemistry*, 67 (2006) 1849.
19 Demir E, Serdar G & Sökmen M, Comparison of some extraction methods for isolation of catechins and caffeine from Turkish green tea. *Int J Second Metab*, 2 (2015)
20 Moore A, Yust-Katz S, Icht O, Eliyahou R, Gordon N, Cohen AY, Goldstein IM, Peled N, Seigal T, Amiel A & Dudnik E, Bevacizumab for stereotactic radiosurgery-induced radiation necrosis in patients with non-small cell lung cancer treated with immune check-point inhibitors. *J Neurol Sci*, 427 (2021) 117556
21 Fujioka K, Salaheldin TA, Godugu K, Meyers HV & Mousa SA, Edible green solvent for optimized Catechins extraction from green tea leaves: Anti-hypercholesterolemia. *J Pharm Pharmacol Res*, 6 (2022) 80.
22 Diwan B & Sharma R, Green tea EGCG effectively alleviates experimental colitis in middle-aged male mice by attenuating multiple aspects of oxi-inflammatory stress and cell cycle deregulation. *Biogerontology*, 23 (2022) 789.
23 Carnazza M, Yang N, Tiwari RK, Geliebter J & Li XM, Natural compounds targeting MAPK, PI3K/Akt, and JAK/STAT signalling pathways in cancer therapy. *Int J Mol Sci*, 26 (2025) 10498..
24 Dong S, Bi H, Zheng D, Li Y, Zhao Y & Peng W, Analysis of biodrugs extracted from kiwi fruit by FT-IR and GC-MS. *J Environ Biol*, 40 (2019) 509.
25 Zhang L, Xie J, Gan R, Wu Z, Luo H, Chen X, Lu Y, Wu L & Zheng D, Synergistic inhibition of lung cancer cells by EGCG and NF-κB inhibitor BAY11-7082. *J Cancer*, 10 (2019) 6543.
26 Madar IH, Sultan G, Chelliah R & Oh DH, Screening for anticancer activity: DNA fragmentation assay. *Methods Actinobacteriol*, (2022) 439.
27 Felordi MS, Alikhani M, Farzaneh Z, Choshali MA, Ebrahimi M, Es HA, Piryaei A, Najimi M & Vosough M, Epigallocatechin-3-gallate induced apoptosis by dissociation of c-FLIP/Ku70 complex in gastric cancer cells. *Sci Rep*, 13 (2023) 19353.
28 Matić IS & Pavić V, Microwave-assisted Extraction of Polyphenols from Plant Materials: A Review. *J Chem Technol Biotechnol*, 93 (2018) 34.
29 Banu KS & Cathrine L, General techniques involved in phytochemical analysis. *Int J Adv Res Chem Sci*, 2 (2015), 25.
30 Wnuk E, Zwolak I & Kochanowicz E, The physiological levels of epigallocatechin gallate (EGCG) enhance the Cd-induced oxidative stress and apoptosis in CHO-K1 cells. *Sci Rep*, 14 (2024) 13625.
31 Pandir D, Protective effect of epigallocatechin-3-gallate on capsaicin-induced DNA damage and oxidative stress in human erythrocyes and leucocytes *in vitro*. *Cytotechnology*, 67 (2015) 367.
32 Shahriari Felordi M, Alikhani M, Farzaneh Z, Alipour Choshali M, Ebrahimi M, Aboulkheyr Es H, Piryaei A, Najimi M & Vosough M, Epigallocatechin-3-gallate induced apoptosis by dissociation of c-FLIP/Ku70 complex in gastric cancer cells. *J Cell Mol Med*, 27 (2023) 2572.
33 Milani AT, Rashidi S, Mahmoudi R & Douna BK, Cytotoxic activity of epigallocatechin and trans-cinnamaldehyde in gastric cancer cell line. *Asian Pac J Cancer Biol*, 4 (2019) 71.

34 Al-Kawaz HS & AL-Mashhedy LAM, Green Synthesis of Silver Nanoparticles Using *Actinidia deliciosa* Extracts. *Res J Pharm Biol Chem Sci*, 7 (2016) 2212.
35 Salama ZA & Zeinab A, Active Constituents of Kiwi (*Actinidia deliciosa Planch*) Peels and Their Biological Activities as Antioxidant, Antimicrobial, and Anticancer Agents. *J Med Plants Res*, 12 (2018) 92.
36 Dong S, Bi H, Zheng D, Li Y, Zhao Y & Peng W, Analysis of biodrugs extracted from kiwi fruit by FT-IR and GC-MS. *J Environ Biol*, 40 (2019) 509.
37 Wnuk E, Zwolak I & Kochanowicz E, The physiological levels of epigallocatechin gallate (EGCG) enhance the Cd-induced oxidative stress and apoptosis in CHO-K1 cells. *Sci Rep*, 14 (2024) 13625.
38 Ghasemi M, Turnbull T, Sebastian S & Kempson I, The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. *Int J Mol Sci*, 22 (2021) 12827.
39 Singh N, Agrawal S, Jiwnani S, Khosla D, Malik PS, Mohan A, Penumadu P & Prasad KT, Lung cancer in India. *J Thorac Oncol*, 16 (2021) 1250.
40 Cheng H, Wang Y, Yang J & Xu W, Methods for Extraction and Purification of Epigallocatechin Gallate from Green Tea: A Review. *Molecules*, 26 (2021) 1005.
41 Yang CS, Wang H & Wang X, Cancer prevention by tea: Evidence and potential mechanisms. *J Nutr*, 148 (2018) 27.
42 Fujioka K, Salaheldin TA, Godugu K, Meyers HV. & Mousa SA, Edible green solvent for optimized Catechins extraction from green tea leaves: Anti-hypercholesterolemia. *J Pharm Pharmacol Res*, 6 (2022) 80.
43 Wu DT, Deng W, Li J, Geng JL, Hu YC, Zou L, Liu Y, Liu HY & Gan RY, Ultrasound-assisted deep eutectic solvent extraction of phenolic compounds from thinned young kiwifruits and their beneficial effects. *Antioxidants*, 12 (2023) 1475.
44 Furukawa A, Oikawa S, Murata M, Hiraku Y & Kawanishi, S, Epigallocatechin gallate causes oxidative damage to isolated and cellular DNA. *Biochem Pharmacol*, 66 (2003) 1769.
45 Pandir D, Protective effect of epigallocatechin-3-gallate on capsaicin-induced DNA damage and oxidative stress in human erythrocyes and leucocytes *in vitro*. *Cytotechnology*, 67 (2015) 367.