

Evaluation of *in vitro* Anti-Cancer Activity of *Bunium persicum* using NCIH-460, A375 and SK-N-SH Cancer Cell Line

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ABSTRACT

Background: The purpose of this research is to determine if *Bunium persicum* have any anticancer effect when tested *in vitro*. However, scientists have not yet fully explored whether this drug can further help in cancer treatment or not. *Bunium persicum* (called Black cummin) is traditionally used for its antioxidant and anti-inflammatory properties. **Aim:** The present study aimed to evaluate *in vitro* anti-cancer activity of *Bunium persicum* extract against Human lung cancer (NCIH-460), Skin melanoma (A375), and Neuroblastoma (SK-N-SH) cell lines. **Materials and Methods:** The *in vitro* anti-cancer activity was evaluated by *Bunium persicum* extract. It could inhibit the growth of Human Lung Cancer (NCIH-460), Skin melanoma (A375) and Neuroblastoma (SK-N-SH) cells in a test tube. Ethanoloic extract of *Bunium persicum* was made and tested for its ability to kill cancer cells in the laboratory. For 24 and 48 hr, cells were exposed to various amounts of the extract (50 µg/mL, 100 µg/mL and so on). The determination of cell viability involved spectrophotometry and the IC₅₀ value was found for every cell line. Changes in the shape of the cells and how they detached from each other were observed using phase-contrast microscopy. **Results:** The present study evaluates the *in vitro* anticancer potential of *Bunium persicum* extract against human lung cancer (NCI-H460), skin carcinoma (A375), and neuroblastoma (SK-N-SH) cell lines. It showed IC₅₀ value on NCIH-460 was 127.72 µg/mL at 250 µg/mL, A375 was 140.47 µg/mL at 250 µg/mL and SK-N-SH Cell was 135.64 µg/mL. The extract exhibited dose-dependent cytotoxicity, significantly reducing cancer cell viability while sparing normal L929 fibroblast cells, as determined by MTT assay and morphological assessment. Phytochemical compounds like terpenoids, flavonoids and phenolics could also be responsible of the observed cytotoxic effect but no experimentally confirmed mechanisms of action (apoptosis or oxidative stress) were observed in this study. Microscopic analysis revealed cytotoxic morphological changes such as cell shrinkage, membrane irregularities, and detachment as observed under phase contrast microscope. While these findings suggest possible apoptotic cell death, based on further validation using caspase activation, Annexin V staining, and gene expression studies is necessary. These results support the potential of *Bunium persicum* as a selective and promising candidate for anticancer therapy. **Conclusion:** *Bunium persicum* caused NCIH-460, A375 and SK-N-SH dose dependent cell death in cell line and the precise mechanism of action, including possible involvement of apoptosis or mitochondrial pathways, requires further experimental confirmation.

Keywords: Dimethyl sulfoxide (DMSO), Dulbecco's modified eagle medium (DMEM), MTT Assay, Nicotinamide adenine dinucleotide hydrogen (NADH), Nicotinamide adenine dinucleotide phosphate hydrogen (NADPH).

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INTRODUCTION

High health burden from cancer ranks as one of the primary causes of death and it leads to major expenses for the ongoing management of affected patients worldwide.¹ In 2020 The International Agency

for Research on Cancer predicted that forty-six cancers together will reach 29.8 million prevalent infections which will create 19.3 million new cancer cases and bring about 10.0 million cancer-related deaths.² According to reports lung cancer stands as the most detected cancer followed by breast cancer and colorectal cancer and others.³ Scientists forecasted that 19.3 million fresh cancer cases would emerge worldwide in 2020 while cancer would lead to 10.0 million fatalities during the same period. Lung cancer proved fatal to most patients while breast cancer and cancer of the colorectal and stomach and liver followed as secondary causes of death.⁴ Earlier research conducted on *Bunium persicum* has been mainly concentrating on the traditional applications



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and the general pharmacological applications of the plant and these are antioxidant, anti-inflammatory, antimicrobial and hepatoprotective effects. Some phytochemical studies have indicated occurrence of the bioactive compounds which include terpenoids, flavonoids, phenolic compounds and essential oils in *Bunium persicum* that are frequently attributed to antioxidant and cytoprotective activity.⁵ There are also limited preliminary studies which have indicated that extracts of *Bunium persicum* might have slight cytotoxicity to a limited number of cancer cell lines, although these studies are restricted in scope, generally only a small number of concentrations are tested, just one cell line is examined, or no comparison is made between the effects in different types of cancers. Also, mechanistic understanding of the potential action of *Bunium persicum* on cancer cell viability, including causing apoptosis, altering oxidative stress, or disrupting tumor-specific signaling, has not been studied. So far, there is no systematic *in vitro* research that has assessed the cytotoxic activity of *Bunium persicum* on the various human cancer models which represent various tissue sources.⁶ This research compares the expression patterns of these four subtypes through qPCR analysis of 12 human lung cancer cell lines.⁷ The current study focuses on three human cancer cell lines, namely: NCIH-460 (lung cancer), A375 (melanoma), and SK-N-SH (neuroblastoma). Those cell lines were chosen to represent epithelial (lung) and melanocytic (skin) and neuroectodermal (neural) cancers, respectively. The NCIH-460 is an established model of non-small cell lung cancer that is common in cytotoxic and mechanistic studies.⁸ A375 is an example of a human melanoma that has a high metastatic capacity that is aggressive. SK-N-SH is a widely used neuroblastoma cell line that can be utilized in assessing anticancer activity of the tumours of neuronal nature. The incorporation of the three different cell lines allows a comparative assessment of the anticancer promise of various tissue sources and biological phenotypes, which gives a wider understanding of the therapeutic applicability of the compound under study.⁹

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals and reagents used in the study were AR grade and sourced from Sigma Aldrich.

Plant Material and Extract Preparation

The fresh aerial parts of *Bunium persicum* were collected from Munsyari district Pithoragarh (7200 ft) in May 2024 and authentically identified by S.K Singh, Botanical Survey of India, Dehradun. *Bunium persicum* aerial parts were dried at room temperature in the absence of light for roughly seven weeks after which they were ground into powder. 10 g of powder were soaked in 100 mL of solvents (absolute ethanol, distilled water) (1:10 w/v sample-to-solvent ratio) for 72 hr at room temperature with frequent agitation to make ethanolic and aqueous extracts. The extracts were then filtered with Whitman filter paper (No.1), then

evaporated to dryness using rotary evaporator under reduced pressure. The crude extract was then collected and stored at comfortable temperature of 20°C (Tables 1-3).

Cell line and Culture conditions

The National Centre for Cell Science (NCCS) in Pune, India provided Human lung cancer (NCIH-460), skin carcinoma (A375) and neuro (SK-N-SH) cell lines. The cell lines required Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin together with 100 µg/mL streptomycin for culture media. The cells grew in a humidified environment at 37°C alongside 5% CO₂ levels. The researchers sub cultured cells when they achieved 70-80% confluence through the usage of 0.25% trypsin-EDTA. All experimental cell populations utilized cells which maintained the logarithmic growth phase. The experimental medium was changed after 48 hr using sterile methods while regularly checking for microbes beneath the microscope. The percentage yield of the extracts was calculated using the formula.¹⁰

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of crude plant material}} \times 100$$

The dried extract was stored at amber colour airtight containers at 4°C under refrigerated condition to avoid degradation of thermolabile and photosensitive compounds then the extract was reconstituted in Dimethyl Sulfoxide (DMSO) for *in vitro* studies.⁸

Cytotoxicity Assays

The laboratory measured *Bunium persicum* extract cytotoxicity against Human Lung Cancer (NCIH-460), skin carcinoma (A375) and neuro (SK-N-SH) cell lines with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cells in 96-well plates with 5×10^3 cells/well concentration required a 24-hr incubation period for adherence. Cells received different concentrations of *Bunium persicum* extract at levels between 12.5 and 400 µg/mL for a period of 24 and 48 hr. Cell viability was determined through absorbance reading at 570 nm of formazan crystals which were extracted with 100 µL of dimethyl sulfoxide (DMSO) after MTT solution (5 mg/mL in PBS) incubation for 4 hr at 37°C. The treatment experiments revealed cell viability results by measuring their percentage compared to untreated control cells. Nonlinear regression analysis through GraphPad Prism software version X.X determined the IC₅₀ values which measure the concentration needed for inhibiting 50% of cell viability. The experiments consisted of three independent runs and the results displayed measurements with Standard Deviation (SD) at their mean.¹¹

ANTICANCER ACTIVITY ON LUNG CELL

Principle

Most research institutions currently depend on tetrazolium salt reduction methods to evaluate cell growth. Cell metabolic activity

resulting from dehydrogenase enzymes reduces yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) which produces reducing agents NADH and NADPH. The internal formazan which forms from this reaction shows colour as purple while spectrophotometry enables scientists to measure its concentration. The assay track both cell proliferation rates together with the decrease in cell viability due to apoptotic or necrotic changes.¹¹

MTT Assay Protocol

Procedure

A centrifugal force of 300×g produced a cell pellet which scientists then suspended in DMEM solution to obtain 10,000 cells in each 200-μl volume. The 96-well plate contained the seeded suspension which received 37°C incubation together with 5% CO₂ atmosphere for 24 hr. Test drugs at different concentrations ranging from 50 to 250 μg/mL were added into wells containing spent medium after incubation for an additional 24-hr period. 100 μl of 10% MTT reagent with a concentration of 0.5 mg/mL was added to the wells after medium aspirating followed by 3 hr of incubation. Then the growth medium was discarded and formazan crystals were dissolved using 100 μl DMSO by performing gentle shaking. The microplate reader utilized to measure absorbance operated at both 570 nm and 630 nm wavelengths (Figures 1-3).^{12,13}

RESULTS

The cytotoxic potential of the plant extract was tested evaluated against NCIH-460 cell line. The plant extract at the dose of

250 μg/mL shows the maximum cell lysis and demonstrated moderate anticancer activity with an IC₅₀=127.72 μg/mL. The dose dependent reduction in viability suggests potential for cancer treatment.

ANTICANCER ACTIVITY ON SKIN CELL

MTT Assay Protocol

Principle

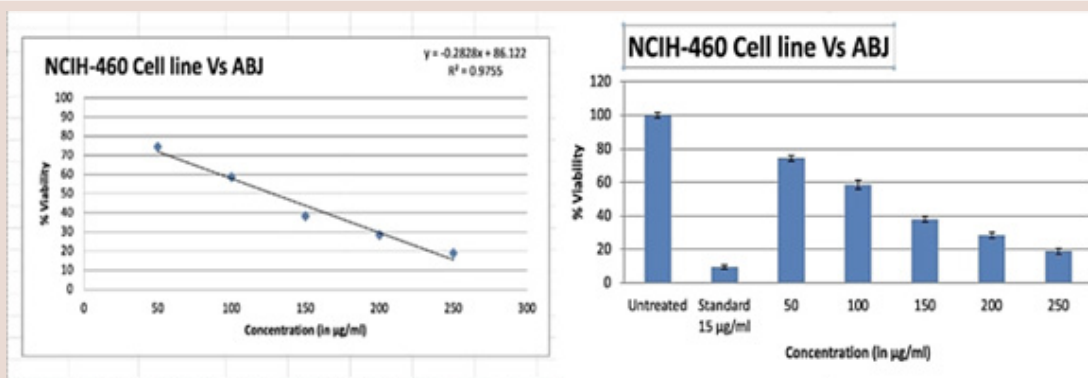
Most research institutions currently depend on tetrazolium salt reduction methods to evaluate cell growth. Cell metabolic activity resulting from dehydrogenase enzymes reduces yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) which produces reducing agents NADH and NADPH.¹⁰ The internal formazan which forms from this reaction shows colour as purple while spectrophotometry enables scientists to measure its concentration. The assay track both cell proliferation rates together with the decrease in cell viability due to apoptotic or necrotic changes.¹¹

Procedure

A centrifugal force of 300×g produced a cell pellet which scientists then suspended in DMEM solution to obtain 10,000 cells in each 200-μl volume. The 96-well plate contained the seeded suspension which received 37°C incubation together with 5% CO₂ atmosphere for 24 hr. Test drugs at different concentrations ranging from 50 to 250 μg/mL were added into wells containing spent medium after incubation for an additional 24-hr period. 100 μl of 10% MTT reagent with a concentration of 0.5 mg/mL was added to

Table 1: Anticancer study of lung test samples.

Concentration (μg/mL)	Mean OD±SD	% Viability
Untreated Control	0.419±0.0092	100
Standard (15 μg/mL)	0.040±0.0064	9.55
50	0.312±0.0106	74.46
100	0.2455±0.0156	58.59
150	0.160±0.0092	38.19
200	0.119±0.0092	28.40
250	0.079±0.0092	18.85



IC₅₀=127.72 μg/mL.

Morphological characterization

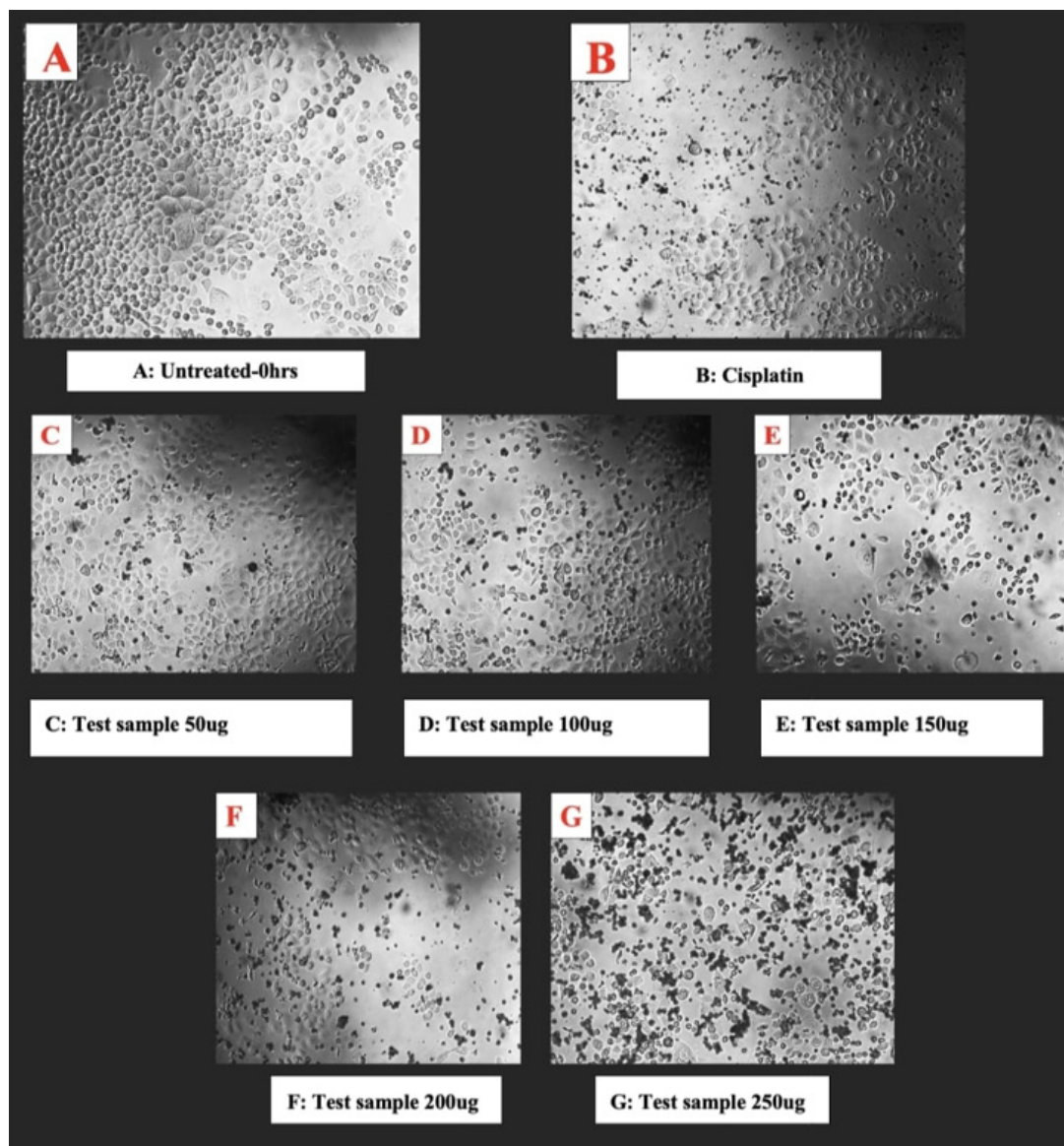


Figure 1: Morphological effects of NCIH-460 untreated cells a) Without treatment b) Standard (cisplatin), Test sample c) ABJ 50 ug, d) ABJ 100 ug, e) ABJ 150 ug, f) ABJ 200 ug, g) ABJ 250 ug of *Bunium persicum*.

the wells after medium aspirating followed by 3 hr of incubation. Then the growth medium was discarded and formazan crystals were dissolved using 100 μ l DMSO by performing gentle shaking. The microplate reader utilized to measure absorbance operated at both 570 nm and 630 nm wavelengths.¹²⁻¹⁵

RESULTS

The cytotoxic potential of the plant extract was tested evaluated against A375 cell line. The plant extract at the dose of 250 μ g/mL shows the maximum cell lysis. The plant extract exhibits dose dependent cytotoxicity against A375 skin cancer cells. Higher concentration (>150 μ g/mL shows, significant cytotoxic effects, suggesting potential anticancer properties. The IC_{50} = 140.47 μ g/mL confirms its moderate effectiveness.

ANTICANCER ACTIVITY ON NEURO CELL

MTT Assay Protocol

Principle

Most research institutions currently depend on tetrazolium salt reduction methods to evaluate cell growth. Cell metabolic activity resulting from dehydrogenase enzymes reduces yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) which produces reducing agents NADH and NADPH. [10] The internal formazan which forms from this reaction shows colour as purple while spectrophotometry enables scientists to measure its concentration. The assay track both cell proliferation rates together with the decrease in cell viability due to apoptotic or necrotic changes.¹¹

Procedure

A centrifugal force of 300×g produced a cell pellet which scientists then suspended in DMEM solution to obtain 10,000 cells in each 200-μl volume. The 96-well plate contained the seeded suspension which received 37°C incubation together with 5% CO₂ atmosphere for 24 hr. Test drugs at different concentrations ranging from 50 to 250 μg/mL were added into wells containing spent medium after incubation for an additional 24-hr period. 100 μl of 10% MTT reagent with a concentration of 0.5 mg/mL was added to the wells after medium aspirating followed by 3 hr of incubation. Then the growth medium was discarded and formazan crystals were dissolved using 100 μl DMSO by performing gentle shaking. The microplate reader utilized to measure absorbance operated at both 570 nm and 630 nm wavelengths.^{12,13}

RESULTS

The cytotoxic potential of the plant extract was tested evaluated against SK-N-SH cell line. The plant extract at the dose of 250 μg/mL shows the maximum cell lysis. The plant extract exhibits dose dependent cytotoxicity against SK-N-SH neuroblastoma cells. Higher concentration (>150 μg/mL shows, significant cytotoxic effects, suggesting potential anticancer properties. The IC₅₀= 135.64 μg/mL confirms its moderate effectiveness.

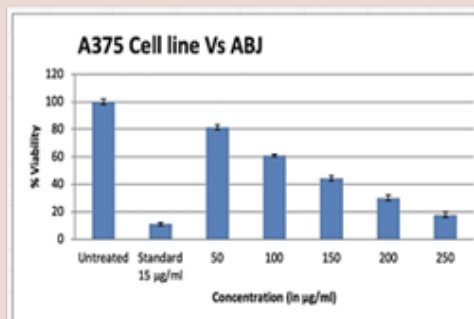
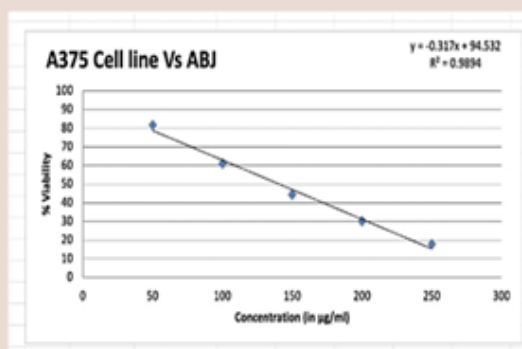
DISCUSSION

The present research investigates *in vitro* anticancer properties of *Bunium persicum* plant extract on Human Lung Cancer (NCIH-460), skin carcinoma (A375) and neuro (SK-N-SH)

cell lines. The research indicates *Bunium persicum* exhibits dose-responsive cytotoxicity that allows this plant extract to harm cancer cells alone without injuring normal cells (L929 fibroblasts) based on both IC₅₀ determinations and cell shape observations. The cytotoxicity observed could be due to the presence of such phytochemicals as terpenoids, flavonoids and phenolic compounds that have been previously described in *Bunium persicum*. Similar phytochemicals have been reported to antioxidant and pro-apoptotic activities in previous studies but in the current study, it was not directly investigated. The cell viability tests performed on MTT showed decreased viability in all cancer cell lines with the best results appearing in the most susceptible cell type. Microscopic examination shows cytotoxic morphological changes, including cell shrinkage and membrane alterations; Similarly phase-contrast microscopy alone cannot definitively confirm apoptosis. The initial morphological observation were consistent and mainly associated with apoptosis but apoptosis was not directly confirmed. According to the previous literature, plant-based compounds were also reported to cause apoptosis by mitochondrial dysfunction and ROS production, but these mechanisms are hypothetical in the current study, with no mechanistic studies conducted. The extract demonstrates promising qualities for discriminating between malignant cells which represents an important criterion necessary for creating secure anticancer treatments. The data indicate that *Bunium persicum* has *in vitro* anticancer potential, but more mechanistic and *in vivo* studies needs to be done before therapeutic conclusions are made. The research conducted only

Table 2: Anticancer study of skin test sample.

Concentration (μg/mL)	Mean OD±SD	% Viability
Untreated Control	0.395±0.0106	100
Standard (15 μg/mL)	0.044±0.0064	11.14
50	0.3225±0.0099	81.65
100	0.241±0.00495	61.01
150	0.175±0.00919	44.30
200	0.119±0.0106	30.13
250	0.0705±0.0113	17.85



IC₅₀= 140.47 μg/mL.

Morphological characterization

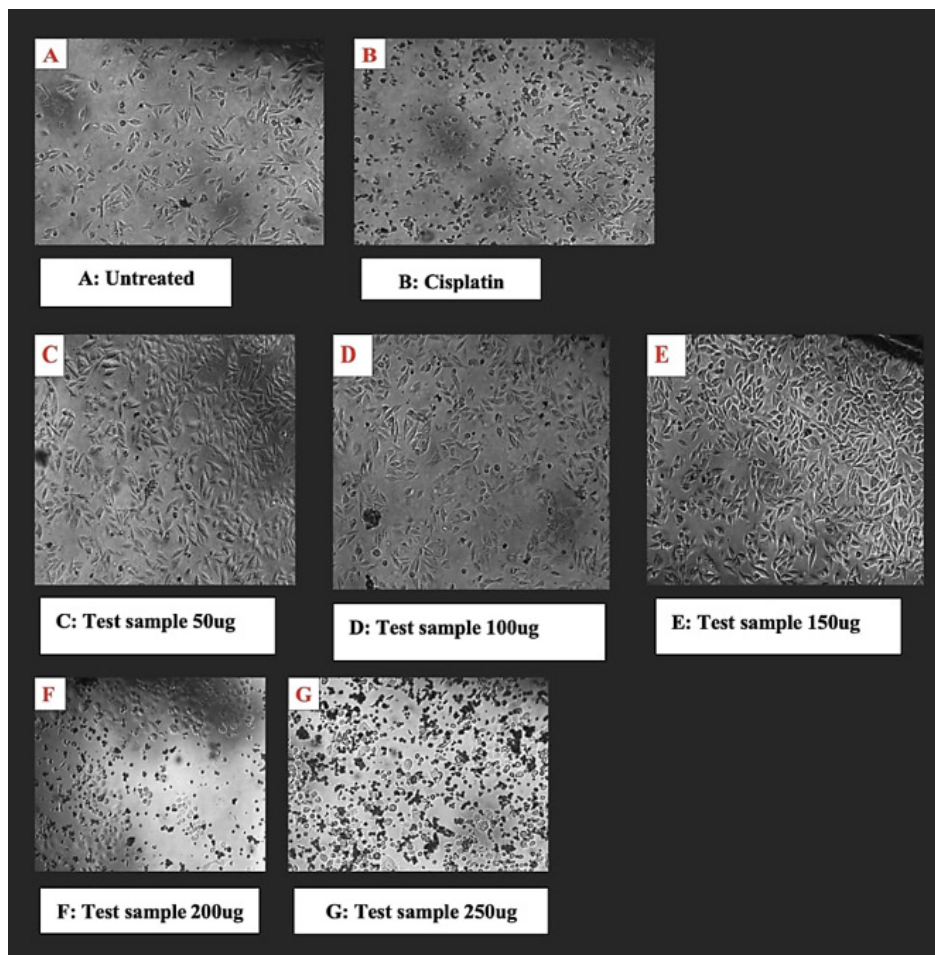
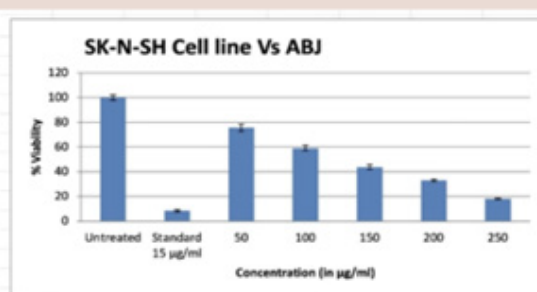
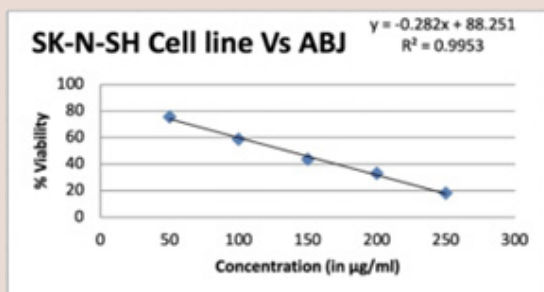


Figure 2: Morphological effects of A375 untreated cells a) Without treatment b) Standard (cisplatin), Test sample c) ABJ 50 ug, d) ABJ 100 ug, e) ABJ 150 ug, f) ABJ 200 ug, g) ABJ 250 ug of *Bunium persicum*.

Table 3: Anticancer study of neuro test samples.

Concentration ($\mu\text{g/mL}$)	Mean OD $\pm$ SD	% Viability
Untreated Control	0.366 $\pm$ 0.0106	100
Standard (15 $\mu\text{g/mL}$)	0.031 $\pm$ 0.00495	8.47
50	0.277 $\pm$ 0.01485	75.68
100	0.216 $\pm$ 0.01061	59.02
150	0.1605 $\pm$ 0.00990	43.85
200	0.121 $\pm$ 0.00354	33.06
250	0.0665 $\pm$ 0.00283	18.17



IC₅₀ = 135.64 $\mu\text{g/mL}$.

Morphological characterization

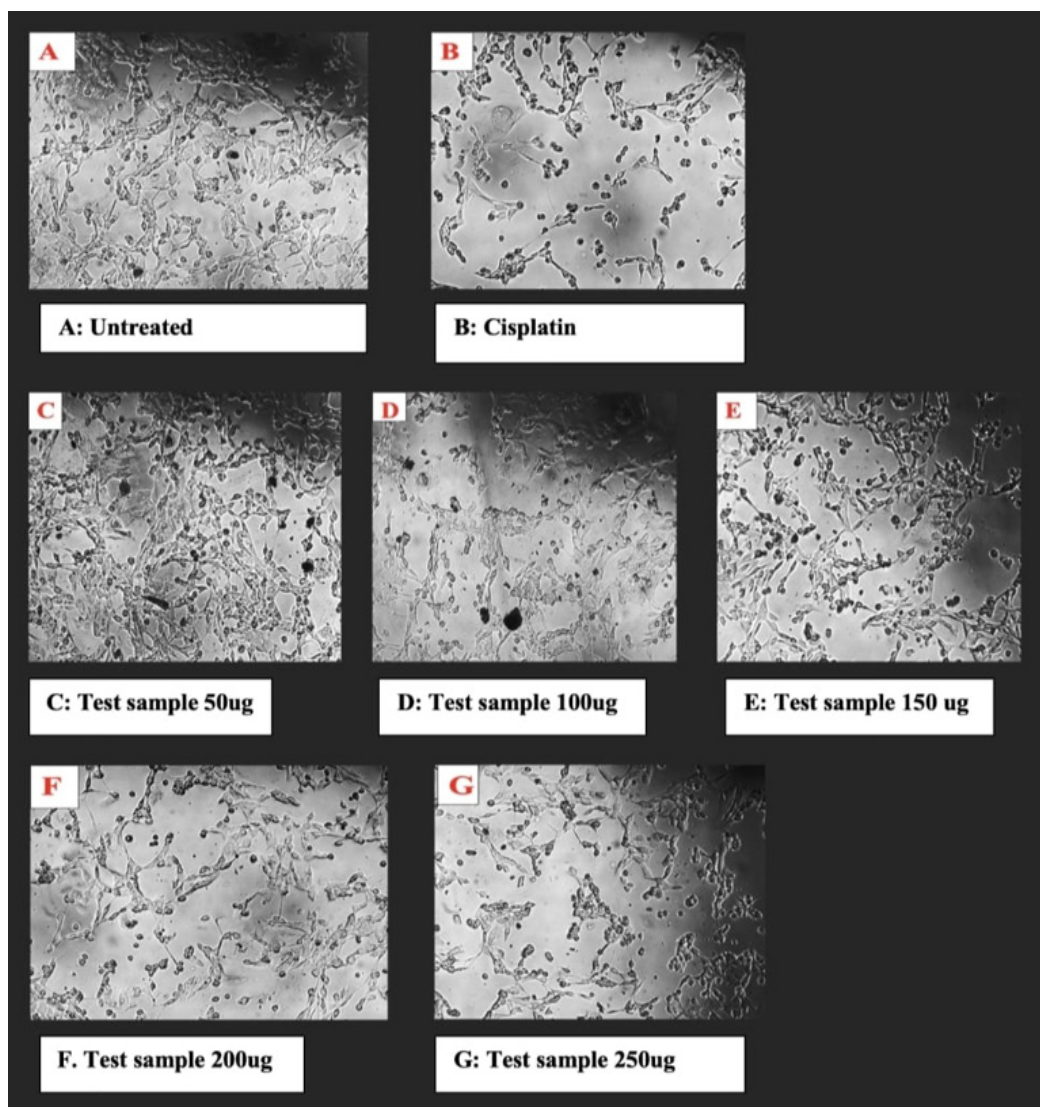


Figure 3: Morphological effects of SK-N-SH untreated cells a) Without treatment b) Standard (cisplatin), Test sample c) ABJ 50 ug, d) ABJ 100 ug, e) ABJ 150 ug, f) ABJ 200 ug, g) ABJ 250 ug of *Bunium persicum*.

used *in vitro* test methods for its analyses. Future studies should focus on:

1. Isolation and characterization of active compounds.
2. Molecular pathway analysis requires Western blotting together with qPCR as detailed methods.
3. Animal testing of anti-cancer drug efficacy under real-world conditions as it pertains to toxicity assessment.

It is necessary to make it clear that the current study was confined to the use of MTT assay and morphological observations of cytotoxicity evaluation. Mechanistic pathways investigated in the study (induction of apoptosis, formation of Reactive Oxygen Species (ROS), and mitochondrial dysfunction) were not experimentally covered. Thus, these mechanisms cannot

be regarded as established conclusions, but propositions or hypothetical explanations of the available literature. Specific biochemical and molecular tests, including caspase activity analysis, Annexin V staining, ROS measurement and mitochondrial membrane potential test, are necessary to determine the mechanism of action in future research.

CONCLUSION

The results of the current study indicate that *Bunium persicum* has a high *in vitro* Anticancer Activity in Human Lung (NCIH-460), neuroblastoma (SK-N-SH), and skin melanoma (A375) cancer cells. The MTT assay demonstrated a clear dose-dependent decrease in cell viability and relative low cytotoxicity was observed in normal L929 cells indicating selective anticancer effect. Morphological observations indicated cytotoxic morphological changes under phase-contrast microscopy, further molecular

assays are required to confirm apoptosis as a mechanism of action. The morphological alterations were noted which might be compatible with the process of apoptosis but they need to be confirmed by molecular techniques including caspase-3 studies, staining with Annexin V, measuring of ROS, and mitochondrial membrane potential.

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ABBREVIATIONS

DMEM: Dulbecco's modified eagle medium; **DMSO:** Dimethyl sulfoxide MTT Assay; **NADH:** Nicotinamide adenine dinucleotide hydrogen; **NADPH:** Nicotinamide adenine dinucleotide phosphate hydrogen; **IC₅₀:** Half-Maximal Inhibitory Concentration.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding this investigation.

SUMMARY

The aim of this research is to determine how *Bunium persicum* acts against three different cancers in human cells, including lung carcinoma, malignant melanoma and neuroblastoma. Aerial part of *Bunium persicum* plant was soaked in methanol for extraction and their cytotoxic activities were checked with the MTT assay. All three cell lines showed a lower rate of growth when treated with the methanolic extract, as compared to the aqueous extract. Out of the three cell lines, NCIH-460 was the most sensitive to the tested compound, as its IC₅₀ level was the lowest. During the analysis, cytotoxic morphological changes such as cell shrinkage and membrane alterations were noted under phase-contrast

microscopy. According to the findings, *Bunium persicum* could be an effective cancer fighter and used as a natural source for medicines aimed at cancer. Additional research will need to identify the active substances in skunk cabbage and understand their way of working.

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