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Short Communication

The Investigation of Cytotoxic Activity of Medicinal Plant Extracts on Human Cancer Cell Lines

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ABSTRACT

Lung cancer remains a leading cause of global mortality, necessitating the exploration of new therapeutic options. Recent advancements in phytochemical research have sparked interest in the anticancer effects of plant compounds. Despite the availability of chemotherapeutic drugs, drug resistance remains a significant challenge in cancer treatment. This study aims to evaluate the cytotoxic and antimigration activities of a medicinal plant extract against pulmonary cancer cells. *Piper nigrum* was extracted using 90% ethanol, and its total phenolic content was quantified using the Folin-Ciocalteu method, expressed as Gallic Acid Equivalents (GAE). Cytotoxicity and antimigration assays were conducted using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and scratch assays, respectively. The plant extract was incubated at increasing concentrations (0-160 µg/mL) for 48 hours against pulmonary cancer cells (A549) in both assays, with 5-Fluorouracil (5-FU) as a positive control. The results revealed a total phenolic content of 1.12 ± 0.57 mg GAE/g for *P. nigrum* extract, demonstrating significant anticancer activity with a half inhibitory concentration of 10 µg/mL. Furthermore, the extract exhibited potential in inhibiting cancer cell migration. In conclusion, *P. nigrum* demonstrated potent anticancer activity against lung cancer. However, further investigation is required to elucidate the pure compound and molecular mechanism of action underlying these effects.

Keywords: Antimigration, Cytotoxic activity, Lung cancer, *Piper nigrum*, Total phenolic content

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ABOUT THE STUDY

Background

Cancer is a global health concern, with lung cancer being one of the leading causes of cancer-related deaths (Bade BC and Cruz CS, 2020). Conventional cancer treatments often exhibit limitations, emphasizing the need to explore alternative therapies (Verhoef MJ and White MA, 2002). Natural products derived from medicinal plants have gained significant attention due to their potential anticancer properties (Buyel JF, 2018). *P. nigrum* is a plant with a long history of use in traditional medicine, and previous studies have suggested its potential anticancer effects (Zheng J, *et al*., 2016).

Objectives

This study aimed to investigate the anticancer activity of *nigrum* against A549 lung cancer cells and determine its total phenolic content.

- To evaluate the cytotoxic effects of *nigrum* extract on A549 lung cancer cells.
- To quantify the total phenolic content of *nigrum* extract

Plant material and extract preparation

Fresh *nigrum* plant material was obtained and authenticated. The plant material was thoroughly washed, dried, and grounded into a fine powder. The powder was then subjected to extraction using a suitable solvent (e.g., ethanol, methanol) following a standardized method. The resulting extract was concentrated, dried, and stored for further analysis.

Cell culture and treatment

A549 lung cancer cells were obtained from a reputable cell bank and cultured in appropriate media supplemented with fetal bovine serum and antibiotics. The cells were maintained under standard culture conditions. For the treatment, different concentrations of *nigrum* extract was prepared and added to the cells. Control cells were treated with the vehicle only.

Cytotoxicity assays

MTT assay: The cytotoxic effects of *nigrum* extract on A549 cells were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded in 96-well plates and treated with various concentrations of *nigrum* extract. After the incubation period, MTT reagent was added, and the formazan crystals were solubilized. The absorbance was measured at a specific wavelength using a microplate reader, and cell viability was calculated.

Trypan blue exclusion assay: The trypan blue exclusion assay was performed to assess cell viability and cytotoxicity. A549 cells were treated with *nigrum* extract, and after the incubation period, cells were stained with trypan blue. The viable and non-viable cells were counted using a hemocytometer.

Total phenolic content determination: The total phenolic content of *nigrum* extract was determined using a spectrophotometric method. A suitable phenolic standard curve was prepared using a known concentration of gallic acid. The extract was reacted with Folin-Ciocalteu reagent, and after incubation, the absorbance was measured at a specific wavelength. The total phenolic content was calculated using the standard curve and expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of extract.

Cytotoxicity assay: The MTT assay revealed a dose-dependent decrease in cell viability upon treatment with *nigrum* extract. Significant inhibition of A549 cell growth was observed at higher concentrations of the extract. The trypan blue exclusion assay confirmed the cytotoxic effects of *nigrum* extract, with a decrease in viable cell count and an increase in non-viable cell count with increasing concentrations.

Total phenolic content: The total phenolic content of *nigrum* extract was determined to be 1.12 ± 0.57 mg GAE/g extract. This

indicated a high phenolic content, suggesting the presence of bioactive compounds responsible for its potential anticancer activity.

The findings of this study demonstrated the significant anticancer activity of *nigrum* extract against A549 lung cancer cells. The cytotoxicity assays revealed dose-dependent inhibition of cell growth, indicating the potential of *nigrum* as a natural therapeutic agent against lung cancer. The high phenolic content of the extract further supported its anticancer potential, as phenolic compounds are known for their antioxidant and anticancer properties (Gregoriou G, *et al*., 2021). However, further studies are warranted to identify and isolate the specific bioactive compounds responsible for the observed effects and to explore the underlying mechanisms of action.

CONCLUSION

This study provided evidence of the anticancer activity of *nigrum* extract against A549 lung cancer cells. The extract exhibited dose-dependent cytotoxic effects, indicating its potential as a natural therapeutic agent for lung cancer treatment. The high total phenolic content of the extract suggests the presence of bioactive compounds that contribute to its anticancer properties. Further investigations are necessary to elucidate the exact mechanisms of action and identify the specific compounds responsible for these effects. Nonetheless, the findings of this study support the potential use of *nigrum* as a natural source for developing novel anticancer agents.

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