

UNVEILING THE ANTICANCER EFFICACY OF CATHARANTHUS ROSEUS: A FOCUS ON A549 LUNG CANCER CELLS

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Abstract

This study aimed to assess the preliminary phytochemical composition and in vitro anticancer properties of Catharanthus roseus leaves against the A549 lung cancer cell line. The phytochemical analysis of the methanol extract was performed using methods similar to those in previous studies. The cytotoxicity of the methanol extract against the A549 cell line was evaluated using the MTT assay to determine cell viability. The analysis identified the presence of alkaloids, flavonoids, saponins, glycosides, tannins, steroids, phytosterols, terpenoids, and phenols. Our findings indicate that the methanol extract of Catharanthus roseus leaves exhibits a significant cytotoxic effect on the A549 cell line in a concentration-dependent manner.

Keywords: *Catharanthus roseus, A549 Lung Cancer Cells, Anticancer Activity, Phytochemical Screening, Methanol Extract, MTT Assay, Cytotoxicity, In Vitro Study, Medicinal Plants, Lung Cancer*

I. INTRODUCTION

The growing interest in herbal medicines stems from a cultural acceptance of natural remedies, alongside a belief in their safety and lower toxicity compared to conventional pharmaceuticals. This trend is underscored by the limitations and side effects often associated with synthetic drugs, prompting both patients and healthcare professionals to seek alternatives [1]. While contemporary drug discovery approaches, such as computer-aided molecular modeling and high-throughput screening, have enhanced our ability to develop new therapeutic agents, the importance of natural products cannot be overstated [2]. Natural compounds derived from plants have been integral to traditional medicine systems and continue to offer valuable insights into novel pharmacological agents [3].

The medicinal efficacy of plants is largely attributed to their phytochemical constituents. Key compounds, including alkaloids, tannins, flavonoids, and phenolic acids, exhibit diverse biological activities, such as antimicrobial, anti-inflammatory, and anticancer

effects [4]. These phytochemicals can disrupt essential life processes in pathogenic microorganisms and cancer cells, providing a basis for their therapeutic application. Furthermore, the strategic use of plant-derived compounds may enhance the efficacy of existing treatments while mitigating adverse side effects associated with synthetic pharmaceuticals, thus promoting a more holistic approach to health management [5].

Cancer, particularly lung cancer, poses a significant public health burden globally. Despite advancements in therapeutic strategies, cancer remains a leading cause of morbidity and mortality. By 2020, it was projected that approximately 15 million new cancer cases would be diagnosed annually, with 70% of these cases occurring in developing countries. Lung cancer is particularly alarming, being the leading cause of cancer-related deaths due to its high incidence and aggressive nature. It is responsible for approximately 17% of all cancers in men and 9% in women, accounting for 19% of all cancer-related fatalities [6]. The primary risk factors for lung cancer include tobacco use, exposure to secondhand smoke, and contact with environmental pollutants and carcinogens, which collectively contribute to its high prevalence and poor prognosis [7].

In light of the pressing need for effective cancer therapies and the rising incidence of lung cancer, research into the anticancer properties of medicinal plants is vital. *Catharanthus roseus*, commonly known as the Madagascar periwinkle, has garnered attention due to its well-documented phytochemical profile and its historical use in traditional medicine. This plant is particularly noted for its alkaloids, such as vincristine and vinblastine, which have been used in the treatment of various cancers. These compounds

inhibit cell division and induce apoptosis in cancer cells, showcasing the potential of *C. roseus* as a source of novel anticancer agents.

In the present study, we aim to investigate the phytochemical constituents of the methanol extract of *Catharanthus roseus* leaves and evaluate its in vitro cytotoxic effects on the A549 human lung cancer cell line. By analyzing the phytochemical composition and assessing the extract's cytotoxicity, we seek to elucidate its potential as a therapeutic option for lung cancer[8]. Our findings may contribute to the development of plant-based treatments that offer safer and more effective alternatives to current cancer therapies. This study not only highlights the significance of herbal remedies in modern medicine but also underscores the necessity of further research into their pharmacological applications.

II. MATERIALS AND METHODS

A. Collection of plant

The whole plant of *Catharanthus roseus* (L.) was collected from Salem district. Leaves were taken for investigation of phytochemical analysis, *in vitro* antioxidant activity and anticancer activity. The leaves were dried in shade dried, coarsely powdered and was extracted with methanol.

B. Preparation of plant extract

Air dried powder was extracted with methanol by using soxhlet apparatus. The residue was filtered and the solvent were evaporated under reduced pressure and the resulted extracts were concentrated using a rotary evaporator with a water bath set at 40°C. The concentrated crude extracts used for further analysis.

C. Preliminary phytochemical analysis:

The qualitative phytochemical analysis of methanol extract of *Catharanthus roseus* leaves were carried out for the presence of alkaloids, flavanoids, saponins, steroids, glycosides, phenols, thiols and resins using the method adopted in similar surveys [9].

In Vitro cytotoxicity of methanol extract of *Catharanthus roseus* against Lung cancer cell lines (A549)

Cell lines: A549 cell line for lung cancer was used for the present study.

Cell culture A549 (human lung carcinoma) cell line were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 u/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO₂ at 37°C.

MTT Assay:

The methanol extract of *Catharanthus roseus* was tested for *in vitro* cytotoxicity, using A549 (human lung carcinoma) cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured A549 (human lung carcinoma) cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10⁵ cells/ml cells/well (200 µL) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the methanol extract of *Catharanthus roseus* in a serum free DMEM medium. Each methanol extract sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO₂ incubator for 24 h.

After the incubation period, MTT (20 µL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) were 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 a micro plate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC₅₀ value was calculated using GraphPad Prism 6.0 software (USA) [10].

III. RESULTS AND DISCUSSION

A. PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary phytochemicals screening of methanol extract of *Catharanthus roseus* was carried out. The methanol extract showed the presence of alkaloids, flavonoids, Saponins, Glycosides, Tannins, Steroids, Phytosterols, Terpenoids, Phenols (Table 1). These phytochemicals are naturally occurring in most plant materials and known to possess various biological activity such as antioxidant, anti-cancer activity, etc, [11].

S.NO	Phytoconstituents	Methanol extract
1	Carbohydrates	-
2	Proteins	-
3	Amino acids	-
4	Alkaloids	+
5	Flavonoids	+
6	Glycosides	+
7	Tannins	+
8	Steroids	+
9	Phyto sterols	+
10	Terpenoids	+
11	Phenols	+
12	Saponins	+
13	Resins	-

Table 1: Phytochemicals screening of methanol extract of *Catharanthus roseus* leaves

B. IN VITRO CYTOTOXICITY TEST:

MTT Assay:

MTT assay was carried out with methanol extract of *Catharanthus roseus* leaves and the results are shown (Figure 1). IC₅₀ value of methanol extract of *Catharanthus roseus* for A549 cells was also calculated and was found to be 240µg/ml. The result of our study revealed that methanol extract of *Catharanthus roseus* has a cytotoxic effect on A549 cell lines in a concentration dependent manner. The highest concentration of the extract (1000µg) inhibited A549 cell growth to maximum level and exhibited higher cytotoxicity which may lead to programmed cell death. Morphological studies also confirmed that the methanol extract of *Catharanthus roseus* leaves has got potential cytotoxic effect.

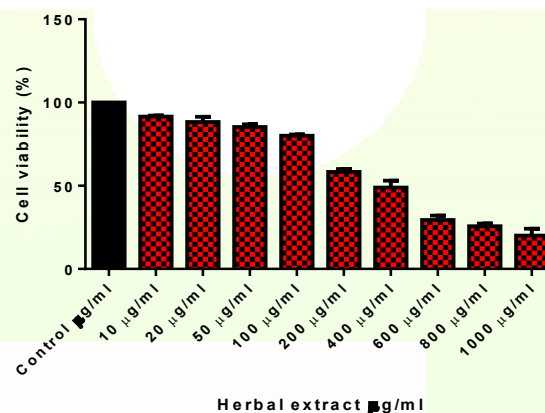


Figure:1 % cell viability of methanol extract of *Catharanthus roseus*

The presence of these secondary metabolites classes is known to have therapeutic activity against several diseases and therefore could suggest its traditional use for the treatment of various illnesses [12]. Flavonoids are one of the most diverse and widespread groups of natural compounds are probably the most important natural phenolics. Flavonoids are a major class of phenolic compounds and are reported in several plant species found to possess many properties including anti-oxidant and anticarcinogenic. Some reports also suggested that most of polar compounds e.g. phenolics and flavonoids are active against reactive oxygen species as well as are cytotoxic[13]. Presence of phenolic compounds and its congeners have shown to induce a cascade based apoptosis in cancer cells, thus inducing cytotoxicity [14].

Lung cancer has the highest death rate in Worldwide exceeding the mortality rates of colorectal, breast and prostate cancers [15]. Incidence and mortality from lung cancer in females is rising while it is declining

in males in developed countries. The majority of lung cancers occur in people who are either current or former smokers. More than 85% of all lung cancer cases occur among people who are either current or former tobacco smokers [16]. The relationship between smoking and lung cancer is caused by the carcinogens present in tobacco smoke. Cancer chemoprevention is rapidly emerging area of preventive oncology that focuses on cancer of the lung prevention by the administration of one or more synthetic or naturally occurring bioactive compounds to repress, reverse or extend the onset of lung tumor [17].

In the present study, MTT assay was conducted to investigate the cytotoxicity of the methanol extract of *Catharanthus roseus* against human lung cancer cell lines. The results of MTT assay showed that the methanol extract of *Catharanthus roseus* has high cytotoxic activity against the cancer cell line from 10µg/mL concentration i.e., 91.53%. At a concentration of 240µg/mL, the methanol extract of *Catharanthus roseus* caused 50% cell inhibition. Polyphenol compounds might inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids could also alter hormone production and inhibit aromatase to prevent the development of cancer cells [18].

The mechanism of action of anticancer activity of phenolics could be by disturbing the cellular division during mitosis at the telophase stage. It was also reported that phenolics reduced the amount of cellular protein and mitotic index, and the colony formation during cell proliferation of cancer cells.

The presence of a 4-carbonyl group of the flavonoid molecule also contributes to anticancer activity. In addition, the presence of 2,3-double bond in flavonoid molecules correlates with mitochondrial damage and cancer cell death [19].

The significant activity of methanol extract of *Catharanthus roseus* can be a result of the induction of cell death by apoptosis as our results indicate. It has been claimed that flavonoids could induce apoptosis in cancer cells. Many work in this direction stressed on inducing apoptosis as a desired strategy of controlling cancers [20]. The overall results indicate the promising baseline information for the potential uses of the methanol extracts of *Catharanthus roseus* as an anticancer agent.

IV. SUMMARY AND CONCLUSION

The study investigated the phytochemical composition and cytotoxic effects of the methanol extract of *Catharanthus roseus* leaves on A549 lung cancer cells. Preliminary phytochemical screening revealed the presence of several bioactive compounds, including alkaloids, flavonoids, saponins, glycosides, tannins, steroids, phytosterols, terpenoids, and phenols, all known for their various biological activities, such as antioxidant and anticancer effects. The MTT assay demonstrated a significant cytotoxic effect of the extract, with an IC₅₀ value of 240 µg/ml and a cell growth inhibition of 91.53% at 1000 µg/ml. These results suggest that the extract may induce programmed cell death in cancer cells, likely due to the action of flavonoids and other phenolic compounds present.

The findings from this study highlight the promising potential of *Catharanthus roseus* as an anticancer

agent, particularly against lung cancer cells. The presence of diverse phytochemicals in the methanol extract correlates with significant cytotoxic effects, suggesting that these compounds could play a critical role in cancer prevention and treatment. Given the high mortality rates associated with lung cancer, further research is warranted to explore the specific mechanisms of action and to evaluate the therapeutic efficacy of *Catharanthus roseus* extracts *in vivo*. This work lays the groundwork for future investigations into the use of natural compounds in oncology and supports the traditional use of this plant in treating various ailments.

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