

Alkaloid-Induced Cytotoxicity of *Plumeria obtusa* in A549 Lung Cancer Cell Line

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Abstract

Cancer continues to be the leading causes of global mortality, with lung carcinoma the most widespread and fatal form; it constitutes a considerable share of cancer mortality worldwide. With growing interest in safer and more cost-effective ways to access cancer treatment, many began to turn their eyes toward plant sources. Among these promising candidates, alkaloids are currently the ones that draw attention because of their actions. *Plumeria obtusa*, belonging from the Apocynaceae family, has long been used in traditional medicine and is known to contain various bioactive phytoconstituents, including flavonoids, triterpenoids, steroids, and alkaloids.

The present work aimed to assess cytotoxic potential of isolated alkaloidal fraction of *Plumeria obtusa* on the A549 human lung carcinoma cell lines. The plant materials (flowers and barks) were sourced locally, authenticated, and systemic extraction was carried out for alkaloid isolation. MTT assay, a colorimetric method that relies on the metabolic conversion of MTT to formazan by viable cells was used to determine the cell viability of extract.

The observations indicated that the alkaloidal extract possessed a concentration-dependent cytotoxic effect. The IC_{50} value was around 96.65 $\mu\text{g/mL}$. Upon treatment with 500 $\mu\text{g/mL}$ and 1000 $\mu\text{g/mL}$ concentrations, the cell viability of A549 cells was declined to approximately 27% and 24%, showing considerable anticancer activity.

This study shows that *Plumeria obtusa* has a high possibility of being exploited as a natural anticancer agent. More work in terms of in vivo studies and mechanism is needed to authenticate its possible usage in future therapy for lung carcinoma.

Keywords: Lung cancer, *Plumeria obtusa*, MTT assay, Alkaloids, A549 human lung carcinoma cell lines

Introduction

The curative parts of medicinal plants are not simply its woody stem or its leaves but the number of chemical compounds (phytoconstituents) it produces and uses for its own growth like flavonoids, alkaloids, gum, mucilage, protein, carbohydrate etc. [1]

Cancer comprises multiple diseases that may arises in any organ of the body, which is defined by the uncontrolled proliferation of abnormal cells. The malignant tumours or neoplasms arise when such cells multiply rapidly, extend beyond their normal boundaries, infect neighbouring tissues, and expand to distant parts of body through a process called metastasis, which is the primary cause of mortality in cancer patients. [2]

The incidence of cancer related to lungs in India is estimated to enhance from 63,708 cases in 2015 to 81,219 cases by 2025, making it a growing public health concern. Increasing incidences are somehow linked to higher rates of smoking and environmental pollution. Around 80- 85% cases are diagnosed at advanced stages, where curative treatment is not possible, leading approximately 60,000 deaths annually. The cases of lung cancer are not evenly distributed and

leading to regional disparities in all over India, Northeast States showing highest cases of lung cancer as per Population Based Cancer Registries Report 2016. With around 130 million smokers, current or ex-smokers, the country has a huge at-risk population, yet the availability of CT scanners is limited due to the burden of tuberculosis complicating screening. Initial studies indicated that low-dose CT scan could have reduced mortality by 13-20%. A practical solution for targeted programs in India would be to combine imaging with AI-based tools and low-cost blood biomarkers to enhance the early detection of lung cancer and its survival rate. [3]

Cancer treatment has evolved into a rapidly expanding field of research. Both traditional and modern approaches are used to combat cancer. Cancer is treated through a range of methods, like chemotherapy, radiation treatment, and surgery. All of them, yet, have some drawbacks. Conventional chemical usage has toxicities and adverse consequences. However, as the issue continues, novel strategies are needed for disease control, especially due to the failure of traditional chemotherapeutic methods. Thus, new approaches to cancer prevention and treatment are required in order to reduce the disease's death rate. Treatment with herbs has emerged as an accessible, safe, and low-toxicity source of bioactive compounds with anticancer potential, owing to the therapeutic potential found in various medicinal plants. [4]

Genus *Plumeria* belonging to Apocynaceae family, contain mainly of flowering trees or shrubs that are cultivated across the tropical region including various parts of India, which are grown as decorative plants. [5] Some species of *Plumeria* with medicinal uses include *P. alba*, *P. accuminata*, *P. lancifolia*, *P. rubra*, *P. phagidenica*, and *P. drastic*. The healing qualities of these species often come from their latex, which can be quite harsh and even corrosive. Various types of *Plumeria* are utilized to treat conditions like rheumatism, diarrhoea, blennorrhoea, venereal diseases, leprosy, psychosis, and issues related to diuresis, among others, are also recognized for their applications in aromatherapy [6] and for exhibiting significant antibacterial and antifungal activities. [7,8]

Despite various parts of the plant demonstrating notable biological activities, there's still been quite a bit of limited research into the anticancer properties of *Plumeria obtusa*. To address this gap, we chose A549 human lung cancer cell lines to investigate the cytotoxic effects of the alkaloidal extracts derived from its flowers and bark. This study seeks to pinpoint the key phytoconstituents found in *Plumeria obtusa* and evaluate the cytotoxic effects of its flower and bark alkaloidal extracts on A549 lung cancer cells in vitro.

Botanical Insights into *Plumeria* Species

Plumeria obtusa, known as the Singapore graveyard flower, is an evergreen ornamental tree whose fragrant flowers yield essential oil used in perfumes. [9] This tree can grow as tall as 8 meters, featuring a rounded canopy and spreading branches. The leaves are glossy green, mildly odorous, sweet in taste, obovate with rounded apices, measuring 60–75 cm in length, smooth in texture, and rigid upon fracture. The flowers are white, strongly fragrant, sweet-tasting, funnel-shaped with a tubular base, about 4.1 cm in size, smooth in texture, and hard when fractured. Typically, propagation is done in the spring with cuttings taken from the leafless tips of stems. [10]

Phytochemical investigations reveal that *Plumeria* species contain iridoid glycosides in addition to alkaloids, tannins, sterols, carbohydrates and triterpenoids. The specific compounds can vary based on the species and the extraction techniques used. The shoots of *Plumeria obtusa* consist of various pentacyclic triterpenoids, such as α -amyrin, kaneroside, and oleandrin, in addition to other special substances. Researchers have isolated two novel iridoids from the foliage of *Plumeria obtusa*: 6"-O-acetylplumieride p-Z-coumarate and 6"-O-acetylplumieride p-E-

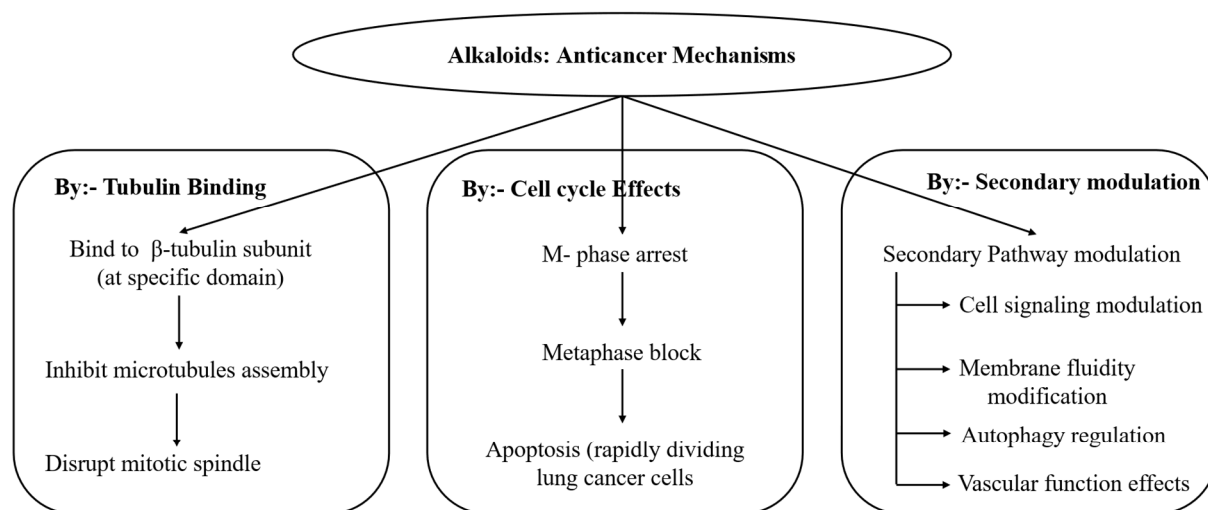


Figure 1: depicts the various mechanisms of action by which alkaloids exhibit anticancer activity.

coumarate. Additionally, they have recognized three specific constituents: plumieride, plumieride p-Z coumarate, and plumieride p-E-coumarate. [11]

Alkaloids exert anticancer effects through mechanisms such as tubulin binding, where interaction with the β -tubulin subunit inhibits mitotic spindle formation and hinders microtubule assembly. The disruption also has an effect on the cell cycle, where it stops cells in the M-phase, causes metaphase blockage, and eventually causes rapidly dividing lung cancer cells to undergo apoptosis. Alkaloids can also operate through secondary processes, which involve modulating several pathways, including vascular function, autophagy regulation, membrane fluidity, and cell signaling, as shown in figure 1. [12]

MATERIAL AND METHOD

Plant material:

We gathered the flowers and bark from *Plumeria obtusa* in Saifai village, located in the Etawah district, to assess the cytotoxic activity of the plant. The identity of plant was confirmed by Professor (Dr.) Rajvir Singh from the Botany Department at KK Degree College in Etawah, Uttar Pradesh.

Extraction:

Extraction of Alkaloids from *Plumeria obtusa*

The collected plant materials were thoroughly washed to remove any dust and impurities from the outside, and then they were dried in the sun. The dried plant material was mechanically ground into a coarse powder.

The coarse powder was treated with petroleum ether in a Soxhlet apparatus to remove the fatty constituents. The defatting process went on until the solvent was clear, indicating that all the fatty impurities had been fully removed. After defatting, the plant residue was treated with a dilute ammonia solution to make the medium alkaline. This helps to extract the alkaloids from their salt forms, converting them into free base forms. The residue that had been treated with ammonia was left to dry fully at room temperature.

The dried alkaline residue was extracted with chloroform using a Soxhlet extractor. The chloroform extract was subjected to rotary evaporation under reduced pressure, resulting in a crude extract. The concentrated chloroform extract was treated with aqueous acid which convert the alkaloids into salt form that are soluble in water and the aqueous layer was separated. To release the alkaloids into their free basic forms, the aqueous acidic layer was then basified once more using ammonia. The alkaloid fraction was separated by re-extraction of this basified solution using chloroform. Concentrate the chloroform extract under reduced pressure, yielding a brownish residue.

Phytochemical Analysis

The initial phytochemical analysis of the residue showed that alkaloids were present, confirmed by standard qualitative tests like Dragendorff's, Wagner's, Hager's, and Mayer's. [13] We then used this final alkaloid extract for in vitro cytotoxicity screening against A549 human lung carcinoma cell lines.

Cytotoxic activity:

In Vitro Cytotoxicity Analysis Using MTT Colorimetric Assay

The cytotoxic activity of the isolated residue was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. The A549 cell line obtained from the National Centre for Cell Sciences (NCCS), Pune was used for cytotoxic screening. 10000 cells per well was cultured for 24 hrs by using the culture media Dulbecco's Modified Eagle medium (AT 149-1 L HiMedia) in 96 wells. The culture media was supplemented with 1% antibiotic solution (Penicillin-Streptomycin Sigma Aldrich P0781) and 10% Fetal Bovine Serum (HiMedia -RM 10432) under controlled condition at temperature 37°C with 5 % CO₂. [14]

After being incubated for 24 hours, the cells were subjected to varying amounts of the alkaloidal extracts, as outlined in Table 1. Untreated cells served as the negative control group, while wells without MTT reagent were considered as blanks. [15]

The plates were further incubated for 2 hours to enable metabolically active cells for the conversion of MTT into formazan crystals. After the incubation period, we gently took out the culture supernatant and dissolved the formazan crystals with 100 µL of dimethyl sulfoxide (DMSO SRL).

Absorbance was measured at 540 nm using an Elisa Plate Reader (iMark, Biorad, USA). [14] We calculated the percentage of cell viability relative to the control group, and with IC₅₀ (half-maximal inhibitory concentration) values obtained through GraphPad Prism software version 6. The results are presented as the average IC₅₀ with the standard error of the mean (SEM). Morphological observations of treated and untreated cells were captured using an Olympus EK2 inverted microscope fitted with a 10 MP Am Scope Aptima CMOS digital camera as shown in Figure 3. [16,17]

Calculation

$$\% \text{ Viable cells} = \left(\frac{A_{\text{test}}}{A_{\text{control}}} \right) \times 100$$

(A_{test} = Absorbance of test sample)

(A_{Control} = Absorbance of Control)

Results and Discussion

Alkaloids are prominent phytoconstituents which shows marked pharmacological activity in small concentration. In the above experiment we have isolated alkaloidal fraction from the plant *Plumeria obtusa*. The cytotoxic activity was screened by invitro MTT assay. It is a colorimetric estimation in which metabolically active cells reduce the yellow tetrazolium dye 3-(4,5dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) into insoluble purple formazan crystals.

S. No.	Sample Conc. ($\mu\text{g/ml}$)	% Viable cells w.r.t. control	SD	SEM	N
1.	0 (control)	100	5.81	2.91	4
2.	1	81.27	5.53	2.77	4
3.	10	72.79	3.65	1.82	4
4.	50	56.89	5.46	2.73	4
5.	100	44.52	6.58	3.29	4
6.	250	36.74	4.85	2.43	4
7.	500	27.21	3.53	1.77	4
8.	1000	24.03	3.36	1.68	4

Table 1- Statistical analysis of Cytotoxic activity

MTT assay results showed that the treatment of A549 cell line was with different concentrations of isolated alkaloidal fraction exhibited cytotoxic activity, with the 50% inhibitory concentration sitting at approximately 96.65 $\mu\text{g/ml}$. The IC₅₀ refers to the concentration at which the cell viability is cut down by half.

Furthermore, at concentrations of 500 and 1000 $\mu\text{g/ml}$, the percentage of viable cells was only about 27% and 24%, respectively, compared to the control. The sample demonstrated significant cytotoxic activity, suggesting it could be a promising candidate for further research as a potential anticancer agent.

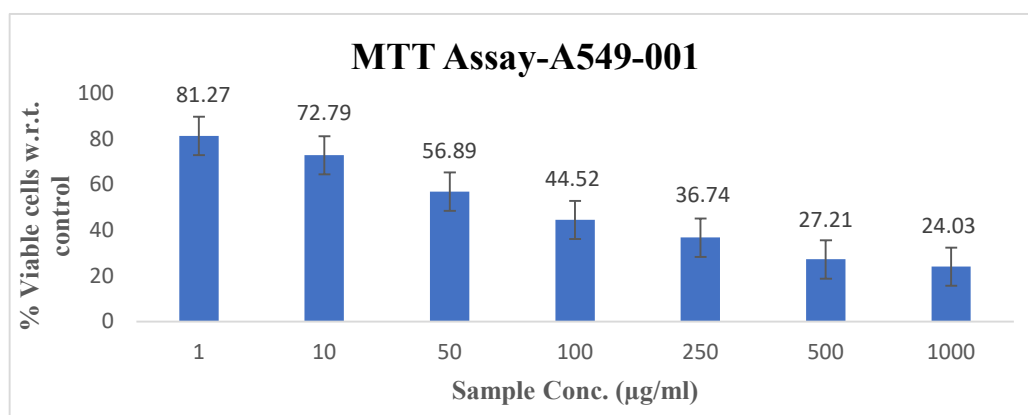


Figure 2: The graph depicts a dose-dependent decrease in A549 cell viability, with sample concentration ($\mu\text{g/ml}$) on the X-axis and % viable cells (relative to control) on the Y-axis.

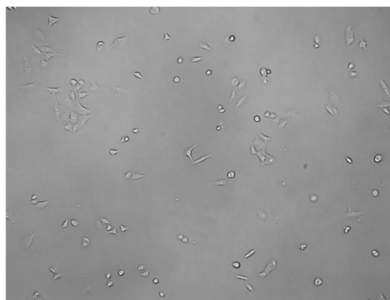
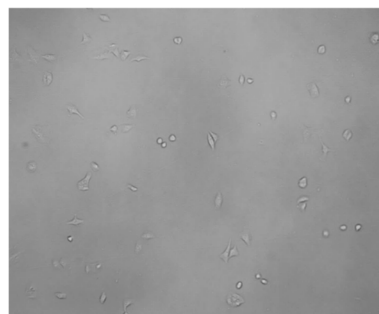
a) Control**b) Treated at 100µg/ml**

Figure 3: Images showing cell line treatments: a) Control b) treatment at 100 µg/mL

Conclusion

Plumeria obtusa, a member of Apocynaceae family contains alkaloids, like other plants of this family which includes Vinca species. These species contain strong anticancer compounds like vincristine and vinblastine; these constituents play a vital role clinically but found in very small quantities. Which makes the extraction very laborious and require lots of plant material. In the present study, the cytotoxic activity is screened for alkaloidal fraction of *Plumeria obtusa*. The results show the significant cytotoxic properties of alkaloidal fraction against A549 human lung carcinoma cell line in in-vitro analysis. The study performed only on invitro mode and further in-depth studies are required to be done on the plant. If the study shows prominent result *Plumeria obtusa* may serve as potent anticancer medicine which is comparatively affordable and having high alkaloidal content. Therefore, further research and scientific validation requires for pharmacological potential of *Plumeria obtuse*.

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