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CYTOTOXIC EFFECTS OF *PHYLLANTHUS NIRURI* ON LUNG CANCER CELLS: AN *IN-VITRO* ANALYSIS

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ABSTRACT

Lung cancer is a leading cause of cancer – related deaths worldwide. A malignant lung tumor, lung cancer or lung carcinoma is defined by unchecked cell proliferation in the lung tissues [2]. The leading cause of death for both men and women in the United States of America (USA) is lung cancer, a highly aggressive, quickly spreading, and common malignancy. The search for effective and non-toxic treatments has led to increased interest in medicinal plants. *Phyllanthus niruri*, a traditional herbal remedy, has shown promising anticancer properties. *Phyllanthus niruri* were extracted using Soxhlet apparatus with ethanol as a solvent. Lung cancer cells were treated with varying concentration of *Phyllanthus niruri* extracts, and cell viability was assessed using MTT assay. The study demonstrates the potential of *Phyllanthus niruri* as a therapeutic agent against lung cancer. Further studies are needed to explore the underlying mechanisms and to evaluate the *in vivo* efficacy of *Phyllanthus niruri* extract.

KEYWORDS

Lung cancer, *Phyllanthus niruri*, ethanol and MTT assay.

I. INTRODUCTION

Lung cancer has been the most prevalent cancer worldwide for a number of decades [1]. A malignant lung tumor, lung cancer or lung carcinoma is defined by unchecked cell proliferation in the lung tissues [2]. The leading cause of death for both men and women in the United States of America (USA) is lung cancer, a highly aggressive, quickly spreading, and common malignancy [3]. Because of the sharp increase in incidence rates and lung cancer fatalities over the past century, which has been linked to rising cigarette usage, the disease has spread like wildfire [4]. Additionally, it discovered that people who kept smoking had a higher death rate [5]. Each year, approximately 2.20 million new cases of lung cancer are identified [6], and 75% of these individuals pass away within five years of receiving their diagnosis [7]. TYPES: Small cell lung cancer (SCLC) accounts for 13% of all lung cancers, while non-small-cell lung cancer (NSCLC) accounts for 84% of all lung cancers [8]. The non-small cell lung cancer (NSCLC) comes in three varieties: a. About 40% of lung malignancies are adenocarcinomas, which typically develop outside the lung. b. Squamous cell carcinoma, which makes up 25% of lung malignancies, typically develops in the middle of the lung, close to the trachea. c. large cell carcinoma, which makes about 10% of lung malignancies, can develop anywhere in the lung [9]. According to the World Health Organization's (WHO) 2021 categorization system, lung cancers are separated into the following categories: Lung neuroendocrine neoplasms, salivary gland-type tumors, neuroendocrine tumors, squamous precursor lesions, squamous cell carcinomas, large cell carcinomas, sarcomatoid carcinomas, precursor glandular lesions, adenocarcinomas, adenosquamous carcinomas, and other epithelial tumors [10]. **EPIDEMIOLOGY:** Male mortality is higher than female mortality in the United States [11]. With an estimated 2 million new cases and 1·76 million deaths annually, lung cancer is the most common cancer diagnosed and the leading cause of cancer-related deaths globally [6]. The primary risk factor for lung cancer is smoking (relative risk [RR] = 10 to 30 when compared to nonsmokers) [12,13]. In 79 percent of males and 90 percent of women, smoking is directly associated with lung cancer [14]. Another risk factor is exposure to secondhand smoke [15,16]. Lung cancer is also frequently caused by environmental and occupational factors, such as exposure to ionizing radiation, vinyl chloride, nickel, chromium, and arsenic [17]. A slight but considerable risk of lung cancer was linked to radon exposure in uranium miners [18]. Lung cancer risk has also been associated with specific dietary components [19,20]. **SIGN AND SYMPTOMS** Lung cancer is extremely heterogeneous, arising in many different sites in the bronchial tree, so depending on its anatomic location, it can present with a wide range of symptoms and signs [21,22]. Numerous epidemiologic studies have suggested that people who consume diets low in beta-carotene are at a higher risk of developing lung cancer [23]. No particular signs and symptoms exist for lung cancer [24]. Cough is prevalent in 50 to 75% of people with lung cancer. Roughly 20 to 40 percent of patients with lung cancer experience chest pain, and up to 25 to 40

percent of individuals may have dyspnea at present [25]. Coughing, hemoptysis, weight loss, exhaustion, fever or clubbing, hypercalcemia, myasthenic syndrome (muscle tiredness), and alterations are typical symptoms [9]. **PATHOPHYSIOLOGY:** The pathophysiology of lung cancer is intricate and poorly understood. It is thought that repeated exposure to carcinogens, like cigarette smoke, causes lung epithelium to dysplasia, which can then result in genetic mutations and protein synthesis [26], which disrupts the cell cycle and encourages carcinogenesis. MYC, BCL2, and p53 for small cell lung cancer (SCLC) and EGFR, KRAS, and p16 for non-small cell lung cancer (NSCLC) are the most prevalent genetic alterations that cause lung cancer development [27,28]. According to research, those who pass away within 90 days of receiving a lung cancer diagnosis had more contacts with their general practitioner before receiving the diagnosis than those who lived longer. This suggests that there may be a lack of awareness causing them to lose earlier opportunities to receive the diagnosis [29]. **DIAGNOSIS** Being one of the most common cancers to be diagnosed, lung cancer can be difficult to identify. The development of tailored treatment and accurate histological and molecular characterizations of lung cancer are closely linked to diagnostic issues [30]. According to evaluations, the campaign resulted in 700 more lung cancer diagnoses than the year before, with about 400 more individuals receiving a diagnosis at an earlier stage [31]. A chest X-ray often begins the work-up, and if it is abnormal, a computer tomographic scan should follow [32]. The UK National Screening Committee is actively considering low dose CT scanning as a lung cancer screening method. In comparison to chest X-rays, it has been demonstrated to be an efficient method of detecting early-stage lung malignancies and improving mortality [33]. Sputum cytology's sensitivity and specificity for lung cancer diagnosis may approach 0.66 and 0.99, respectively, in skilled hands [34]. Depending on the tumor's location, additional diagnostic testing may be necessary. For central tumors, a bronchoscopy is the most effective method since it provides for direct visualization of the tumor and airways while also obtaining biopsy material for histologic diagnosis [32]. Unwelcome Staging Confirming the staging and histological differentiation of cancer, as well as obtaining tissue or pathologic confirmation of malignancy, comes next after CT and PET scans [35]. The gold standard for diagnosing and staging lung cancer used to be mediastinoscopy. It is primarily used to sample lymph nodes following the negative needle approach and in cases when the patient's lymph node size or FDG uptake on a PET scan indicate that they are still at high risk for malignancy [36]. The use of endoscopic ultrasonography (EUS) in the diagnosis and staging of lung cancer is growing [37]. **TREATMENT:** Therefore, the existence or absence of adenocarcinoma, a person's ethnicity, gender, smoking history, and gene mutation must all be taken into consideration when choosing a treatment [38]. For individuals with non-small cell carcinoma in stages I through IIIA, surgery is the preferred course of treatment [39]. Surgical resection rates have risen from 9% to over 17% in the last decade [40]. Preoperative chemotherapy may increase survival in individuals with non-small cell carcinoma, according to recent evidence. Adjuvant chemotherapy is the norm for patients having total resection without any prior treatment [41]. In the treatment of lung cancer, specialist palliative care is equally essential, and significant effort has been made to maximize its application and enhance patient outcomes [42]. Pembrolizumab, nivolumab, and atezolizumab are immune checkpoint inhibitors, the newest family of systemic therapies [33]. Brachytherapy and other endoscopic treatments assist manage symptoms brought on by airway narrowing [43,44]. **PREVENTION:** Promoting smoking cessation is arguably the primary care physician's most significant role in preventing lung cancer. The most successful cessation therapies (with quit rates ranging from 16 to 21 percent) include structured telephone counseling, bupropion (Wellbutrin), nicotine replacement, and nortriptyline [45-53]. When combined with social or behavioral support, the quit rate can rise to 35 percent [54]. It has also been demonstrated that doctors' informal counseling can somewhat raise quit rates [55,56]. Nonspecific, but not COX-2–specific, COX inhibition, farnesyltransferase inhibition, glucocorticoids, 5-lipoxygenase inhibition, prostacyclin synthase overexpression, a prostacyclin analog (iloprost), and other tactics have all been used to successfully prevent chemoprevention in mice [57].

Phyllanthus niruri

The Amazon rainforest and other tropical regions, such as South East Asia, Southern India, and China, are home to the small, upright annual herb P. niruri, one of the Phyllanthus species, which can reach a height of 30 to 40 cm. Its alternating, sessile, oblong leaves range in length from 7 to 12 cm [58]. The oblate, veiny, reticulate, stramineous capsules have a diameter of around 3 mm. The stipules are quite sharp. Monoecious P. niruri [59]; In South and Southeast Asian traditional medicine, Phyllanthus niruri, a perennial tropical shrub, has been used to treat a variety of ailments, such as kidney stones, genitourinary infections, jaundice, diarrhea, and dyspepsia [60]. The leaves and fruit have been used to cure gallstones and jaundice in traditional medical systems including Ayurvedic and Unani medicine [61]. The herb, known as Bhumyamalaki in South India, is thought to cure syphilis, gonorrhea, and constipation [62]. This herb, known locally as "pitirishi," has become well-known in northern India as a home cure for bronchitis, asthma, and even tuberculosis [63]. This herb's young shoots might occasionally be used as an infusion to treat persistent diarrhea [64]. Classification in botany: Phyllanthus niruri Class: Magnoliopsida; Order: Euphorbiales; Family: Euphorbiaceae; Genus: Phyllanthus; Species: Niruri; Kingdom: Plantae; Division: Magnoliophyta [65].

Phytochemistry

Class	Compounds
Alkaloid	4-Methoxy-nor-securinine, nirurin, ent-norsecurin[66]
Lipids	Ricinoleic acid[66]
Sterol	Estradiol, Beta sitosterol [66]
Benzenoid	Gallic acid[67]
Coumarin	Ellagic acid, ethyl brevifolin carboxylate[67]
Flavonoid	Lignin Phyllanthin[69], phyltetralin,nirtetralin, niranthin[70], hypophyllanthin [69]
Tannin	Geranin[71]

Pharmacological activity

1. Antioxidant and hepatoprotective activity[72]
2. Antidiabetic – hypoglycaemic action[73]
3. Anti-inflammatory, antinociceptive and analgesic activity[74]
4. Hypolipidaemic activity [75]
5. Cardioprotective activity[76]
6. Antiplatelet and vasorelaxant activity[77]
7. Wound healing and anti ulcer activity[78]
8. Antiviral activity [79]
9. Antibacterial activity [80]
10. Antineoplastic activity [81]
11. Immunomodulatory activity [82]

II.MATERIALS AND METHODS:

SOXLET EXTRACTION:

Soxhlet extraction is a very useful tool for preparative purposes in which theanalyte is concentrated from the matrix as a whole or separated from particular interfering substances. Sample preparation of environmental samples has been developed for decades using a wide variety of techniques. Solvent extraction of solid samples, which is commonly known as solid–liquid extraction (also referred to as leaching or Lixiviation in a more correct use of the physicochemical terminology), is one of the oldest methods for solid sample pretreatment. Conventional Soxhlet extraction remains as one of the most relevant techniques inthe environmental extraction field. **Materials Required** Ethanol was purchased from Merk, USA. Whatman No.1 filter paper was purchased from Millipore, USA. **Procedure** Test sample (PN07) can be fresh or dried. It needs to be crushed, using a pestle andmortar, to provide a greater surface area. The test sample should be sufficient to fill the porous cellulose thimble (in ourexperiments we use an average of 14 g of thyme in a 25- x 80-mm thimble). All equipment should be too assembled. Build a rig using stands and clamps tosupport the extraction apparatus. Following this, the Ethanol is added to a round bottom flask, which is attached to aSoxhlet extractor and condenser on an isomantle. The crushed plant material is loaded into the thimble, which is placed inside theSoxhlet extractor. The side arm is lagged with glass wool. The solvent is heated using the isomantle and will begin to evaporate, movingthrough the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and thecycle begins again. The process should run for a total of 4 hours. Once the extraction set up, it can be left to run without direct supervision. It is not advised to leave the equipment completely alone due to the mix of runningwater and an electrical appliance, so a technician or other lab user should be madeaware. The equipment can be turned off.

Fig 1: Soxhlet extraction

BIOLOGICAL STUDIES:

Material required 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), DMEM medium, 1X PBS, (India). 96 well tissue culture plate and wash beaker were from Tarson (India). **PRINCIPLE** MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) assay, is based on the ability of a mitochondrial dehydrogenase enzyme of viable cells tocleave the tetrazolium rings of the pale yellow MTT and form a dark blue colored formazan crystal which is largely impermeable to cell membranes, thus resulting inits accumulation within healthy cells. Solubilization of cells by the addition Of detergents (DMSO) results in the liberation of crystals which are solubilized. The number of surviving cells is directly proportional to the level of formazan product created. The color can be quantified using a multi-well plate reader. **PROCEDURE: Cell culture** A549 (Human Lung cancer cell line) were purchased from NCCS, Pune andwere cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum(FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under anatmosphere of 5% CO_2 at 37ºC**. MTT assay** The Test sample was tested for *in vitro* cytotoxicity, usingA549 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 1×10^5 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 hour at 37°C. The wellswere washed with sterile PBS and treated with various concentrations of the Test sample in a serum-free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. 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 underan inverted microscope. Finally, the medium together with MTT (220 µL) was aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolveformazan 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 microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value were calculated using Graph Pad Prism 6.0 software (USA).

Formula Cell viability % = Test OD/Control OD X 100

III.RESULT AND DISCUSSION:

BIOLOGICAL STUDIES

Table 1: OD Value at 570 nm

S. No.	Tested sample concentration (µg/ml)	OD value at 570 nm(in triplicates)		
1	Control	1.312	1.351	1.366
2	500 µg/ml	0.567	0.572	0.635
3	400 µg/ml	0.665	0.67	0.681
4	300 µg/ml	0.801	0.804	0.826
5	200 µg/ml	0.835	0.836	0.848
6	100 µg/ml	0.859	0.864	0.896
7	80 µg/ml	0.896	0.913	0.927
8	60 µg/ml	0.943	0.948	0.969
9	40 µg/ml	0.977	1.019	1.029
10	20 µg/ml	1.133	1.134	1.139
11	10 µg/ml	1.245	1.192	1.205

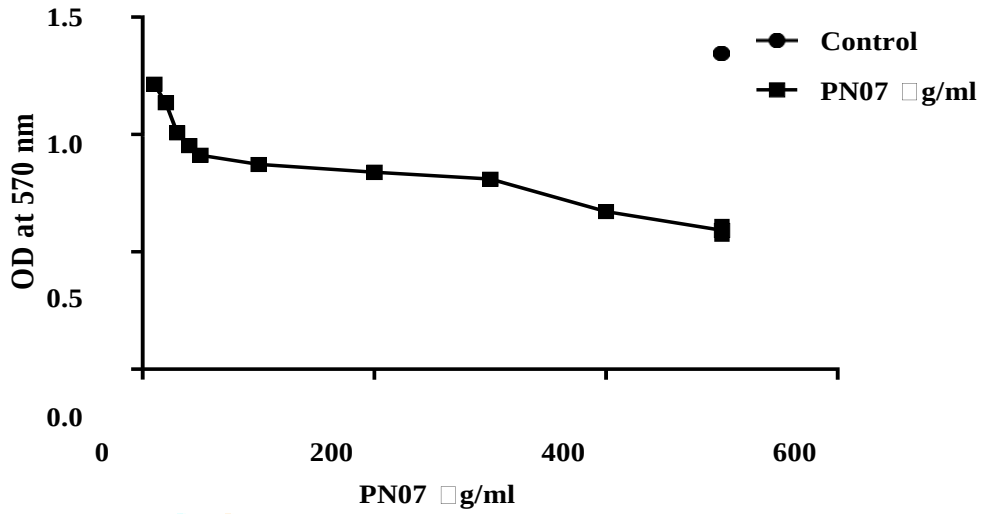
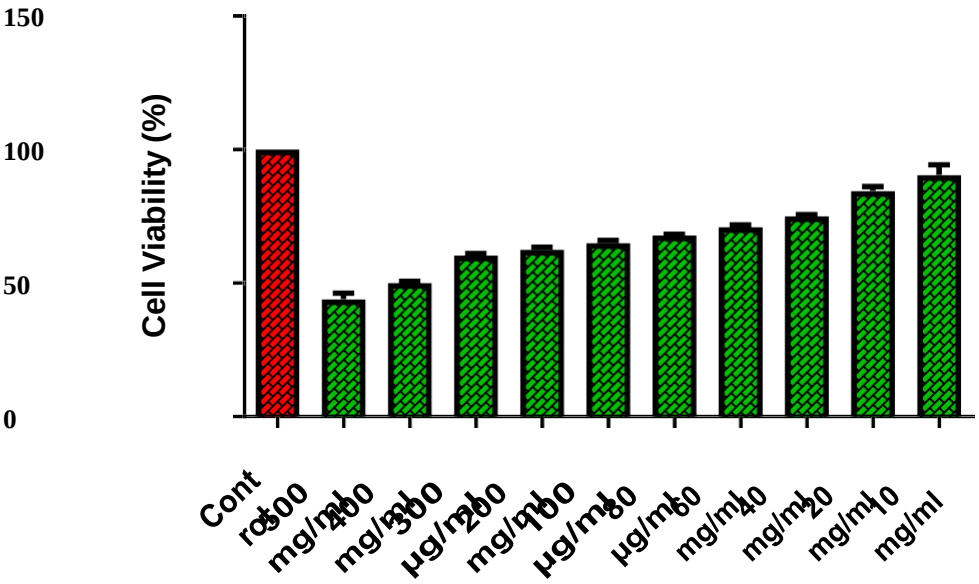


Table 2: Cell Viability (%)

S. No.	Tested sample concentration (µg/ml)	Cell viability (%) (in triplicates)			Mean Value (%)
1	Control	100	100	100	100
2	500 µg/ml	43.2165	42.339	46.4861	44.013854
3	400 µg/ml	50.686	49.5929	49.8536	50.044152
4	300 µg/ml	61.0518	59.5115	60.4685	60.343941
5	200 µg/ml	63.6433	61.8801	62.0791	62.534148
6	100 µg/ml	65.4726	63.9526	65.593	65.006054
7	80 µg/ml	68.2927	67.5796	67.8624	67.911542
8	60 µg/ml	71.875	70.1702	70.937	70.994096
9	40 µg/ml	74.4665	75.4256	75.3294	75.073834
10	20 µg/ml	86.3567	83.9378	83.3821	84.55889
11	10 µg/ml	94.8933	88.2309	88.2138	90.445999

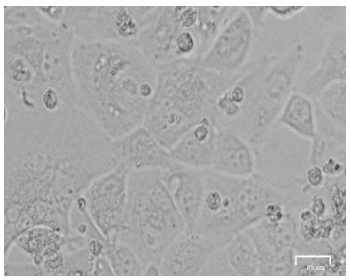


PNO7 g/ml

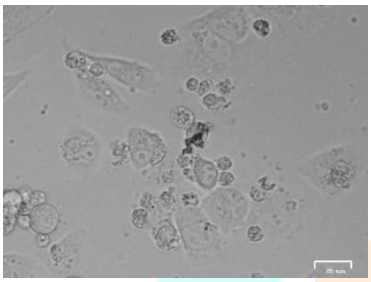
Table 3: IC50 Value of tested sample: 79.10 µg/ml

log(inhibitor) vs. normalized response --Variable slope	
Best-fit values	
LogIC50	1.898
HillSlope	-0.9754
IC50	79.10
Std. Error	
LogIC50	0.04454
HillSlope	0.09836
95% CI (asymptotic)	
LogIC50	1.807 to 1.989
HillSlope	-1.177 to -0.7739
IC50	64.11 to 97.59
Goodness of Fit	
Degrees of Freedom	28
R squared	0.8848
Sum of Squares	2952
Sy.x	10.27
Number of points	
# of X values	30
# Y values analyzed	30

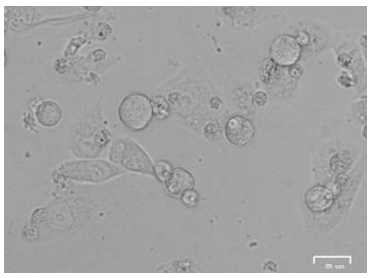
Controlled cell



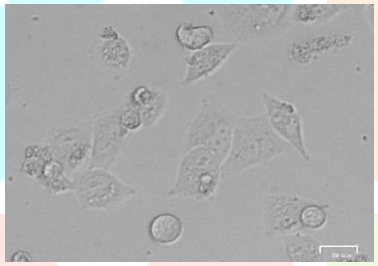
PN07 500 µg/ml



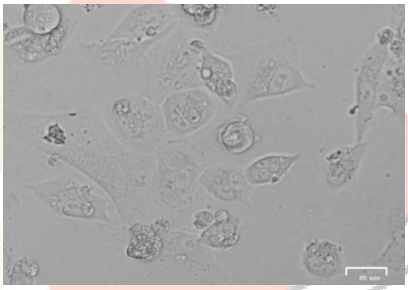
PN07 300 µg/ml



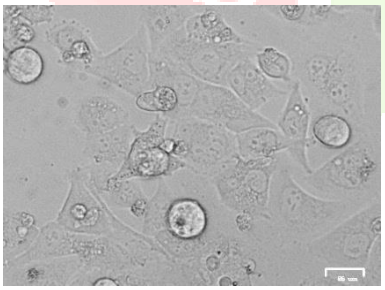
PN07 100 µg/ml



PN07 60 µg/ml



PN07 20µg/ml



PN07 10 µg/ml

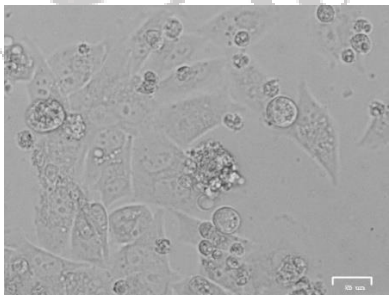


Fig 2: Images of control cells and treated cells.

The present study aimed to investigate the cytotoxic effects of PN07 on cell growth and viability. The results showed that PN07 exhibited a concentration-dependent inhibitory effect on cell growth, with an IC50 value of 79.10 µg/ml. This suggests that PN07 has potential cytotoxic effects, which is consistent with previous studies on similar compounds. The cell viability assay results showed that PN07 significantly reduced cell viability at higher concentrations (500-100 µg/ml), indicating strong cytotoxicity. In contrast, lower concentrations (60-10 µg/ml) exhibited moderate cytotoxicity. These findings suggest that PN07's cytotoxic effects are dose-dependent, and higher concentrations may be more effective in inhibiting cell growth. The images from the cell morphology study (Fig. 2) showed visible changes in cell density and morphology upon treatment with PN07, further supporting the cytotoxic effects observed in the cell viability assay. The mechanisms underlying PN07's cytotoxic effects are not fully understood and require further investigation. However, it is possible that PN07 may interact with cellular components, such as DNA or proteins, to inhibit cell growth and induce cytotoxicity.

FUTURE DEVELOPMENT:

1. **Mechanistic Studies** – Future research should focus on elucidating the precise molecular mechanisms underlying PN07's cytotoxic effects. This includes exploring its role in apoptosis induction, cell cycle arrest, oxidative stress, and modulation of key signaling pathways involved in cancer progression.
2. **Bioactive Compound Identification** – Isolation and characterization of the active phytochemicals in PN07 responsible for its cytotoxic properties will be crucial. Advanced analytical techniques such as HPLC, LC-MS, and NMR should be employed to identify and quantify these bioactive compounds.
3. **Formulation Development** – To enhance the bioavailability and stability of PN07, novel drug delivery systems such as nanoparticles, liposomes, or polymer-based carriers should be explored. These formulations could improve targeted delivery, reduce toxicity, and enhance therapeutic efficacy.
4. **In Vivo Studies** – Preclinical animal studies are necessary to evaluate the efficacy, pharmacokinetics, and safety of PN07 in living systems. Such studies will help determine the optimal dosage, potential side effects, and long-term effects of treatment.
5. **Clinical Translation** – If preclinical results are promising, clinical trials should be conducted to assess the safety, tolerability, and efficacy of PN07 in human subjects. These studies will be critical in determining its potential as a viable therapeutic agent in cancer treatment.
6. **Combination Therapy Potential** – PN07 should be investigated for its potential synergistic effects with existing chemotherapeutic agents or natural compounds to enhance efficacy and reduce side effects. Combination therapy strategies could help overcome drug resistance and improve treatment outcomes.
7. **Application Beyond Cancer** – Given the traditional medicinal uses of *Phyllanthus niruri*, further research should explore the potential of PN07 in treating other diseases such as viral infections, liver disorders, and inflammatory conditions.
8. **Toxicological and Safety Assessments** – Long-term toxicity studies and safety evaluations should be conducted to ensure that PN07 does not cause adverse effects in non-cancerous cells and tissues.

By addressing these areas, PN07 could emerge as a promising natural therapeutic agent with broad applications in modern medicine.

IV. CONCLUSION

The present study provides compelling evidence that PN07 exerts concentration-dependent cytotoxic effects on cell growth and viability. The observed decrease in cell proliferation suggests that PN07 may possess bioactive compounds capable of inhibiting cancer cell growth, making it a promising candidate for further exploration in cancer therapy. These findings align with previous reports on the pharmacological properties of *Phyllanthus niruri*, highlighting its potential as a natural therapeutic agent.

Despite these promising results, several critical aspects remain to be investigated. Future studies should focus on elucidating the precise molecular mechanisms underlying PN07-induced cytotoxicity, including its effects on apoptotic pathways, cell cycle regulation, and potential involvement in oxidative stress responses. Additionally, in vivo studies are essential to confirm its efficacy, bioavailability, and safety profile in animal models before considering clinical applications.

In summary, PN07 demonstrates significant potential as a natural anticancer agent. However, comprehensive mechanistic studies and preclinical evaluations are necessary to determine its therapeutic applicability and establish its role in modern medicine.

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9. A Review on Lung Cancer with Emphasis on Current Treatment Gayatri Deshmukh*1, Dr. H. A. Sawarkar2 and Dr. K. R. Biyani.2 1Department of Pharmacology, Anuradha College of Pharmacy, Chikhli, Buldhana, Maharashtra, India1 2Campus Director, Anuradha College of Pharmacy, Chikhli, Buldhana, Maharashtra, India2
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