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Phytochemical Analysis and In Vitro Anticancer Activity of Chloroform Extract of Centella Asiatica Leaves Using Lung Cancer (A549)

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ABSTRACT

Medicinal plants are a cornerstone of traditional medicine and are extensively studied for their therapeutic properties. Among them, *Centella asiatica* is well known for its diverse pharmacological benefits, including its potential anticancer properties. This study aims to evaluate the phytochemical composition and in vitro anticancer activity of chloroform extract derived from the leaves of *C. asiatica* against the lung cancer cell line A549. The phytochemical screening revealed the presence of key bioactive compounds such as alkaloids, flavonoids, glycosides, carbohydrates, and amino acids, which are known for their biological and therapeutic effects.

The anticancer potential of the chloroform extract was assessed using the MTT assay, which measures cellular viability by evaluating mitochondrial activity. The extract demonstrated significant dose-dependent cytotoxicity against the A549 cell line, with IC50 values indicating its potency. The results highlight the potential of *C. asiatica* to inhibit cancer cell proliferation and induce cytotoxicity, making it a promising candidate for further development as a natural anticancer agent.

Phytochemicals such as flavonoids and alkaloids, which were detected in the extract, have been extensively reported in the literature for their roles in inducing apoptosis, preventing metastasis, and exhibiting antioxidant activity. These properties likely contribute to the extract's anticancer effects observed in this study. Additionally, the presence of glycosides and amino acids may support complementary mechanisms in targeting cancer cells.

The findings of this study underscore the potential of *C. asiatica* as a natural source for anticancer agents, particularly in the context of lung cancer. Future studies should focus on isolating and characterizing specific active compounds, determining their precise mechanisms of action, and conducting in vivo experiments to validate their efficacy. This research also emphasizes the importance of medicinal plants as a sustainable and accessible resource for drug discovery, offering an alternative to synthetic drugs, which often have significant side effects.

1. INTRODUCTION

Medicinal plants, also known as medicinal herbs, have played a vital role in traditional medicine practices since ancient times. These plants synthesize an array of bioactive compounds for defense against environmental stressors like pathogens, insects, and herbivores. Phytochemicals, the biologically active secondary metabolites in these plants, possess numerous therapeutic properties, including antibacterial, antiviral, antifungal, and anticancer activities (Ahn K, 2017). However, the effects of whole-plant therapies remain uncertain due to the diversity of their chemical constituents, and rigorous scientific evaluations are necessary to establish their efficacy and safety.

In developing countries, medicinal plants are a cornerstone of primary healthcare, largely due to their accessibility and cost-effectiveness compared to synthetic drugs. The global market for medicinal plants reached a valuation of $2.2 billion in 2012, highlighting their widespread usage (Traffic.org, 2017). These plants not only contribute to healthcare but also support rural livelihoods, especially among women, in environmentally sustainable ways (Sharma A, 2017). Traditional systems like Ayurveda, Unani, and Siddha in India underscore the longstanding value of medicinal plants, which are recognized for their potential to act as drug candidates due to their pharmacological properties (Shakya AK, 2016).

The Himalayas host a vast repository of medicinal plant species, with about 17% of India's 15,000 flowering plant species being medicinally significant. Notably, the Indian Himalayan region is home to 1,745 such species, some of which are endemic to Uttarakhand (Prakash R, 2015). Ancient texts, including the *Rigveda* and *Ramayana*, document the use of medicinal plants in healthcare, signifying their historical and cultural importance (Quran S, 2015).

Medicinal plants are also pivotal in modern drug discovery, with 80% of synthetic drugs originating from natural products (Bauer & Brönstrup, 2014). Despite this, only 6% of the estimated 250,000–400,000 plant species have been studied for their biological activity, emphasizing the need for further exploration (Shibu et al., 2012). Studies have shown that plant- derived compounds like alkaloids, flavonoids, and terpenoids exhibit potent anticancer activities by inducing apoptosis and preventing metastasis (Kennedy DO, 2011). The World Health Organization (WHO) estimates that 80% of the population in developing countries relies on traditional medicine for their healthcare needs, further illustrating the significance of phytotherapy (WHO, 2013).

Cancer, a leading global health challenge, often resists conventional treatments like chemotherapy and radiation. Medicinal plants offer a promising alternative for cancer management due to their ability to target cancer cells selectively without causing toxicity (Kaur R et al., 2011). This study investigates the phytochemical composition and anticancer potential of *Centella asiatica*, a well-known medicinal plant, against lung cancer cell lines.

Aim and Objectives

This study aims to evaluate the phytochemical composition and in vitro anticancer activity of the chloroform extract derived from the leaves of *Centella asiatica*. This is one of the pioneering investigations focusing on the anticancer potential of *C. asiatica* against lung cancer cell line A549, adding to the growing body of evidence supporting the therapeutic applications of medicinal plants.

Objectives

- **Collection and Processing**: Gather *C. asiatica* leaves from local sources, followed by cleaning, drying, and powdering for further use.
- **Extraction**: Isolate the crude extract using chloroform as a solvent via Soxhlet extraction to obtain bioactive components.
- **Phytochemical Analysis**: Conduct a systematic analysis of the extract to identify key phytochemicals such as alkaloids, flavonoids, glycosides, carbohydrates, and amino acids.
- **Anticancer Evaluation**: Assess the in vitro cytotoxicity of the chloroform extract against A549 lung cancer cells using the MTT assay to evaluate its potential as a natural anticancer agent.

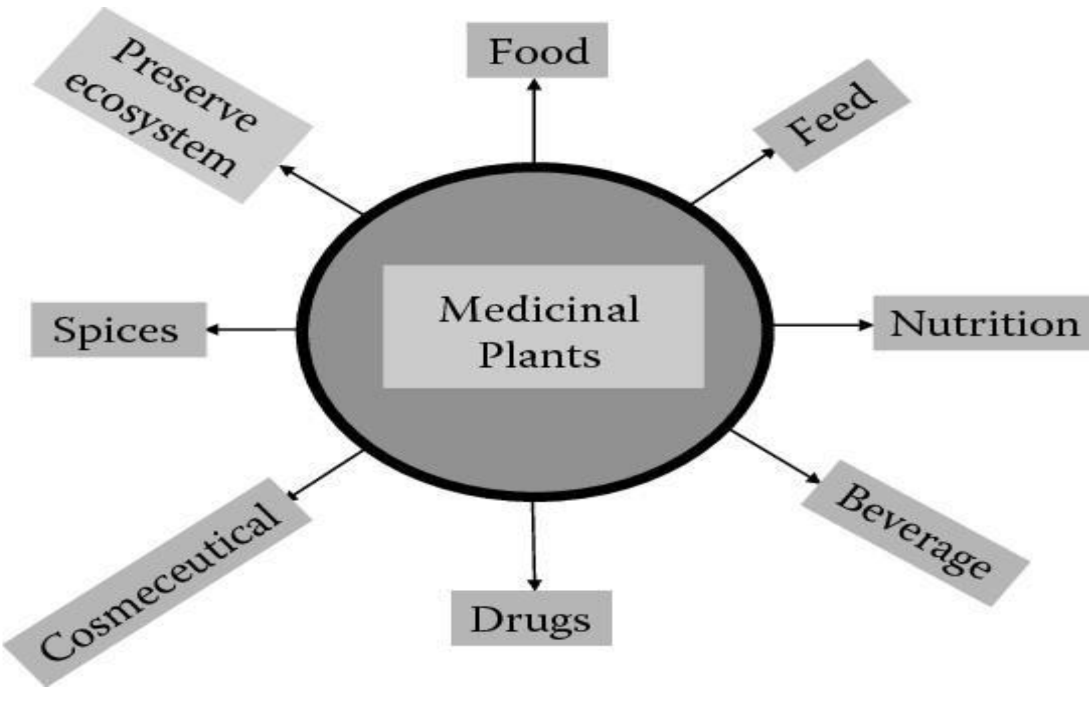

Fig 1.classification of medicinal plants

Phytochemicals and Their Therapeutic Roles

- Phytochemicals, or phytonutrients, are nonnutritive bioactive compounds found in plants that offer protective and disease-preventive properties.
- These compounds include secondary metabolites such as flavonoids, tannins, terpenoids, saponins, and glycosides, which contribute to the plant's defense mechanisms and therapeutic potential (Ngoci Njeru et al., 2013).
- Epidemiological studies suggest a strong inverse correlation between fruit and vegetable consumption and the risk of chronic diseases, including cardiovascular diseases, cancer, diabetes, and osteoporosis (Chen L et al., 2007).
- Phytochemicals help mitigate these diseases by acting as antioxidants, anti- inflammatory agents, and hormonal regulators (Temple et al., 2003).
- **Antioxidants**: Protect cells from oxidative stress, reducing cancer risks (Chen L et al., 2007).
- **Anti-inflammatory**: Suppress inflammation, lowering risks of chronic conditions (Aggarwal BB et al., 2009).
- **Antibacterial**: Provide alternative treatments for infections by targeting bacterial cell walls (Sagbo IJ et al., 2017).
- **Hormonal Modulation**: Isoflavones mimic estrogens, alleviating osteoporosis symptoms (Chen Z et al., 2019).
- **Antiadhesive**: Prevent pathogen adhesion to cell walls, enhancing immune defense (Sagbo IJ et al., 2017).
- Studies involve extracting and isolating plant compounds, determining their structures, and testing biological activities through in vitro and in vivo models (Price et al., 1992).

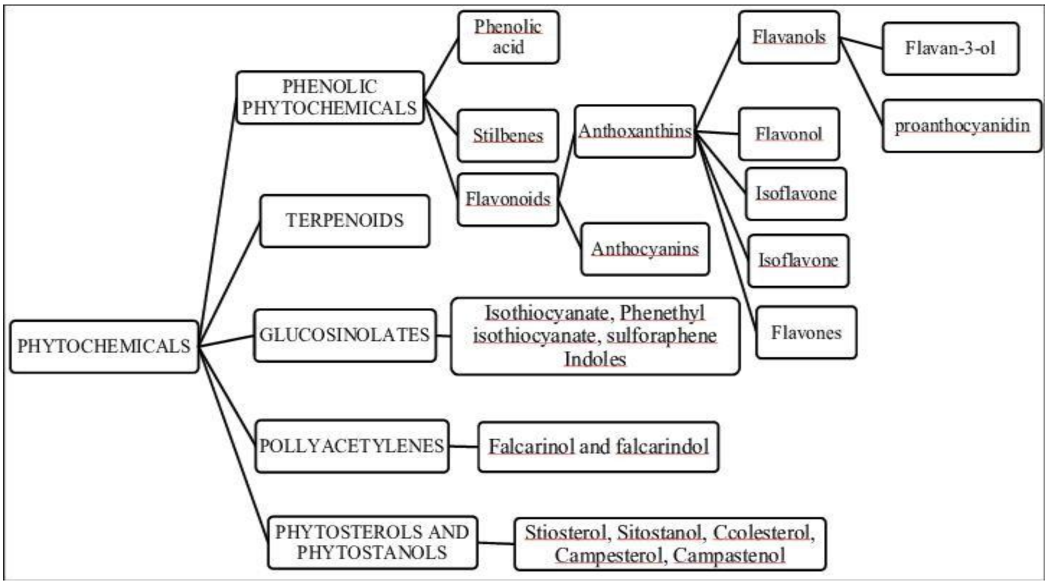

Fig 2. Classification of phytochemicals

Phytochemical Classes and Their Key Features

Phytochemical	Key Features	References
Alkaloids	Plant-derived compounds with antimicrobial properties; neuroactive and therapeutic roles, including anticancer effects (e.g., vincristine and vinblastine).	Matsuura H., et al. (2015).
Flavonoids	Polyphenolic antioxidants found in fruits and vegetables; known for anti-inflammatory, anticancer, and cardioprotective properties.	Panche AN., et al. (2016).
Saponins	Steroid or triterpenoid glycosides with roles in cholesterol reduction, immune stimulation, and anticancer activity.	Desai S., et al. (2009).
Glycosides	Molecules where sugars are bound to non-sugar substances; play roles in cardiac therapy (e.g., digitalis) and hydrolysis	Mondal S. (2019).

Phytochemical	Key Features	References
	processes.	
Steroids	Biomolecules involved in stress response, immune regulation, and inflammation; analyzed for therapeutic and performance-enhancing uses.	Rister AL., et al. (2020).
Terpenoids	Derived from isoprene units; critical for plant defense and human therapeutic applications, including anti-inflammatory and antimicrobial effects.	Rodríguez E., et al. (2007).
Phenolics	Found in plant-based foods; associated with antioxidant and chronic disease prevention effects.	Paul E. (2016).
Tannins	Polyphenolic compounds with antimicrobial, antioxidant, and industrial applications (e.g., food processing, cosmetics).	Chauhan A., et al. (2018).
Resins	Hydrocarbon secretions valued for adhesive properties and use in incense and perfumes; historically significant substances (e.g., frankincense).	Ferrer-Gago FJ., et al. (2019).
Thiols	Sulfhydryl compounds essential in antioxidant defense, detoxification, and apoptosis regulation; decreased levels linked to chronic diseases.	Prakash M., et al. (2009).
Carbohydrates	Primary energy source for metabolism; form structural components (e.g., cellulose) and play roles in protein and lipid associations.	Khowala S. (2008).
Amino Acids	Building blocks of proteins; essential for protein synthesis and metabolic functions, with dietary deficiencies linked to diseases.	Azad S., et al. (2017).
Proteins	Essential macromolecules for tissue repair, enzyme function, and muscle development; requirements vary with age and activity levels.	Moore DR. (2015).

Cancer:

Cancer encompasses a diverse group of diseases characterized by uncontrolled cell growth with the potential to invade or spread to other parts of the body. Unlike benign tumors, malignant tumors can metastasize, leading to widespread disease. Common signs and symptoms include lumps, unexplained weight loss, persistent cough, and changes in bowel or bladder habits.

However, these symptoms can also result from conditions other than cancer.

The term "cancer" originates from the ancient Greek word "καρκίνος," meaning crab, due to the resemblance of some tumors to a crab's shape. Greek physicians Hippocrates and Galen noted this similarity, and the term was introduced into English medical terminology around 1600.

There are over 100 types of cancer, each with distinct characteristics and treatment approaches. All cancer cells exhibit certain hallmarks, including:

- Unregulated cell growth

- Resistance to cell death
- Unlimited replication potential
- Induction of blood vessel formation
- Tissue invasion and metastasis

These features are essential for the development of malignant tumors.

Risk factors for cancer include smoking, obesity, excessive alcohol consumption, poor diet, lack of physical activity, and exposure to certain infections and environmental factors. Preventive measures involve lifestyle modifications such as maintaining a healthy weight, engaging in regular exercise, consuming a balanced diet rich in fruits, vegetables, and whole grains, limiting alcohol intake, avoiding tobacco use, and protecting skin from excessive sun exposure.

Vaccinations against specific infections, like the human papillomavirus (HPV), can also reduce the risk of certain cancers.

Early detection through screening is crucial for improving survival rates in cancers such as cervical and colorectal cancers. The benefits of screening for breast cancer remain a topic of debate. Treatment modalities typically include surgery, chemotherapy, radiation therapy, and targeted therapies, often used in combination. Palliative care plays a vital role in managing symptoms and enhancing the quality of life for patients with advanced cancer.

Globally, cancer incidence and mortality rates have been rising. In 2023, the American Cancer Society estimated that nearly 2 million new cancer cases would be diagnosed in the United States, resulting in about 610,000 deaths. This marks a significant increase from previous years, highlighting the growing burden of cancer worldwide.

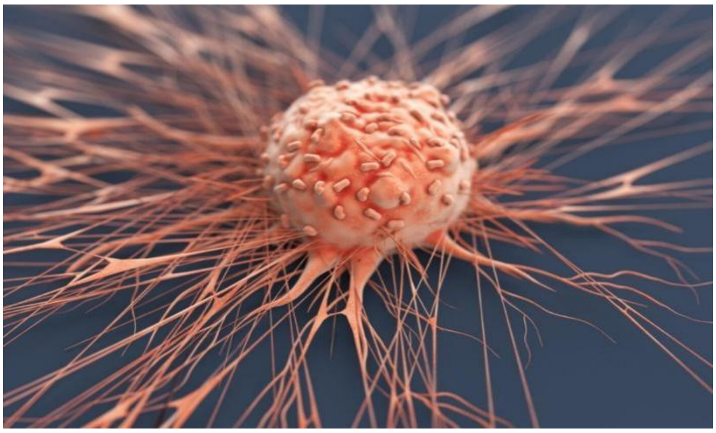

Fig 16. Structure of cancer cells MEDICINAL PLANTS: THEIR USE IN ANTICANCER TREATMENT

Aspect	Details
Cancer Overview	Cancer is characterized by uncontrolled cell growth, leading to the formation of malignant tumors that can metastasize. It is a global health challenge, with research focusing on cures and preventive therapies. (Ochwang'I DO et al., 2014)
Use of Medicinal Plants	Herbal medicines, especially in developing countries, are used for cancer treatment. Terrestrial plant extracts are being researched for potential cancer nanomaterial-based drugs. Plants have natural antiseptic properties that contribute to their medicinal value. (Sivaraj R et al., 2014)
Epigenetic Properties	Cancer development is linked to epigenetic alterations such as hypermethylation of tumor-suppressor genes, which leads to gene silencing.

Aspect	Details
	Drugs that reverse these epigenetic changes are under development. (Schnekenburger M et al., 2014)
Plant Compounds with Anticancer Properties	Plant compounds such as polyphenols, brassinosteroids, and taxols have demonstrated anticancer properties. Many plants have been used traditionally for health benefits, and some are now being researched for their cancer-fighting properties. (Rajeswara Rao BR et al., 2007)
Plant-Derived Anticancer Drugs	Plant-derived drugs, such as sulforaphane, isothiocyanates, and taxanes, show anticancer activity through mechanisms like inhibition of microtubule dynamics and DNA damage. These compounds are being investigated for their cytotoxicity in cancer cell lines and non-toxicity to normal cells. (Amin A et al., 2009; Khazir J et al., 2014)
Medicinal Plant Demand	Medicinal plants, especially from developing regions, are in high demand. Over-exploitation due to informal trade and rapid deforestation threatens plant populations. Sustainable practices, such as utilizing all plant parts and implementing conservation methods (e.g., cryopreservation), are critical for the future. (Parveen S et al., 2013)
Conservation Strategies	Conservation of medicinal plants is essential due to the risk of extinction. Methods like germplasm conservation, cryopreservation, and tissue culture are vital for maintaining biodiversity. These practices can also support industrial use in developed nations. (Zschocke S et al., 2000)
Cytotoxicity of Plant-Derived Drugs	Plant-derived drugs like sulforaphane and taxanes target cancer cell proliferation and promote apoptosis. These compounds, including vinca alkaloids, also reduce cancer cell replication by disrupting microtubules. They offer a non-toxic, natural alternative for cancer treatment. (Pledgie-Tracy A et al., 2007)

Treatment of Non-Small Cell Lung Cancer (NSCLC)

- **Stage I**: Surgery (lobectomy or pneumonectomy) is the main treatment. For those who cannot tolerate major surgery, wedge resection or segmentectomy can be done. No benefit from adjuvant radiation or chemotherapy.
- **Stage II**: Surgery followed by chemotherapy. For tumors invading the chest wall, en-bloc resection is recommended. Pancoast tumors are treated with neoadjuvant chemotherapy and radiotherapy followed by resection.
- **Stage III**: Heterogeneous group with varying treatment. Stage IIIA with N1 nodes can be treated with surgery and chemotherapy. Stage IIIA with N2/N3 nodes, concurrent chemo- radiotherapy is recommended. T4 tumors may require chemoradiation or surgery for specific cases.
- **Stage IV**: Incurable, with treatment focused on symptom management. Chemotherapy may provide small survival benefits, with only 1%-3% surviving 5 years.

Centella Asiatica (Gotu Kola)

- **Botanical Info**: Centella Asiatica, commonly known as Gotu Kola, is a medicinal herb from the Apiaceae family. It grows in wetlands and is native to Southeast Asia and the Indian subcontinent.
- **Uses**: Known for its anti-inflammatory, anti-rheumatic, antibacterial, and antiviral properties. It is used to treat gastrointestinal issues, skin disorders, and as a memory enhancer. It is also used to improve collagen formation, heal burns, and reduce inflammation.
- **Cultural Significance**: In Asia, it has long been used in traditional medicine as a rejuvenator and cure for

various ailments. The plant thrives in shady, damp environments like wetlands and riversides.

- **Skincare Benefits**: Improves wound healing, enhances collagen, reduces inflammation (e.g., in eczema), and has been praised for its ability to heal burns and hypertrophic wounds. Tigers in the wild also use it for self-healing.

Phytochemical Analysis Detection of Alkaloids

- **Mayer's Test**: Yellow precipitate with Potassium Mercuric Iodide → Presence of alkaloids.
- **Wagner's Test**: Brown/reddish precipitate with Iodine in Potassium Iodide → Presence of alkaloids.
- **Dragendroff's Test**: Red precipitate with Potassium Bismuth Iodide → Presence of alkaloids.
- **Hager's Test**: Yellow precipitate with Picric Acid → Presence of alkaloids.

Detection of Flavonoids

- **Alkaline Reagent Test**: Yellow color → Flavonoids present.
- **Lead Acetate Test**: Yellow precipitate → Flavonoids present.
- **Shinoda Test**: Magenta color with magnesium and HCl → Flavonoids present.

Detection of Phytosterols

- **Libermann-Burchard Test**: Bluish-green color with concentrated sulfuric acid → Phytosterols present.
- **Salkowski Test**: Brown ring with sulfuric acid → Phytosterols present.

Detection of Glycosides

- **Modified Borntrager's Test**: Rose-pink color with ammonia → Anthranol glycosides present.
- **Bromine Water Test**: Yellow precipitate → Glycosides present.
- **Keller Killiani Test**: Bluish-green top layer and reddish-brown bottom layer → Cardiac glycosides present.

Detection of Carbohydrates

- **Molisch's Test**: Purple ring with alpha-naphthol and H2SO4 → Carbohydrates present.
- **Fehling's Test**: Brick red precipitate → Reducing sugars present.
- **Benedict's Test**: Brick red precipitate → Reducing sugars present.

Detection of Amino Acids

- **Ninhydrin Test**: Pink or purple color → Proteins, peptides, or amino acids present.

Detection of Tannins

- **Lead Acetate Test**: Precipitate formation → Tannins present.

Detection of Phenols

- **Ferric Chloride Test**: Dark bluish-black color → Phenols present.

Detection of Coumarin

- **NaOH Test**: Yellow color → Coumarin present.

Detection of Quinones

- **Sulfuric Acid Test**: Blue-green or red color → Quinones present.

Detection of Saponins

- **Froth Test**: 1 cm foam layer → Saponins present.
- **Foam Test**: Persistent foam for 10 minutes → Saponins present.

Detection of Terpenoids

- **Salkowski Test**: Reddish-brown color at interface → Terpenoids present.

Detection of Diterpenes

- **Copper Acetate Test**: Emerald green color → Diterpenes present.

Detection of Fixed Oils and Fats

- **Spot Test**: Oil stain on filter paper → Fixed oils/fats present.

Detection of Protein

- **Xanthoproteic Test**: Yellow color with nitric acid → Protein present.

Detection of Resins

- **Acetic Anhydride Test**: Purple color → Resins present.

Detection of Thiols

- **Nitroprusside Test**: Magenta color → Thiols present.

Anticancer Activity

Source of Chemicals and Reagents

- Chemicals purchased from Sigma Aldrich and Hi Media Laboratories Pvt. Ltd.

Cell Culture Maintenance

- **Cell Lines**: MDA-MB-231 (Breast Cancer), A549 (Lung Cancer).
- **Medium**: DMEM with 10% FBS, penicillin (100 U/ml), streptomycin (100 µg/ml), maintained in 5% CO2 at 37°C.

MTT Assay

- **Principle**: MTT is reduced by viable cells, forming a purple formazan product. Measured at 500-600 nm.

Materials and Methods

Collection and Preparation of Plant Extract

Mature *C. asiatica* leaves were collected from a local market, shade-dried, powdered, and extracted using chloroform in a Soxhlet apparatus. The concentrated extract was obtained through rotary evaporation.

Phytochemical Screening

Preliminary qualitative tests were conducted to detect bioactive compounds such as alkaloids, flavonoids, glycosides, carbohydrates, amino acids, resins, and terpenoids using established chemical

protocols.

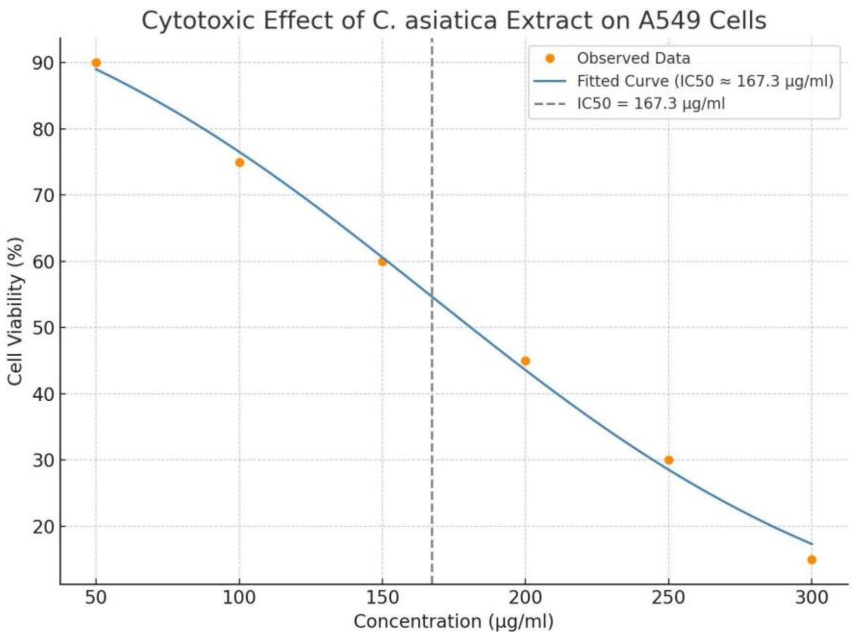

Figure 1: the cytotoxicity curve of *C. asiatica* extract on A549 lung cancer cells, showing the dose-dependent decrease in cell viability and the estimated IC_{50} (~175 µg/ml).

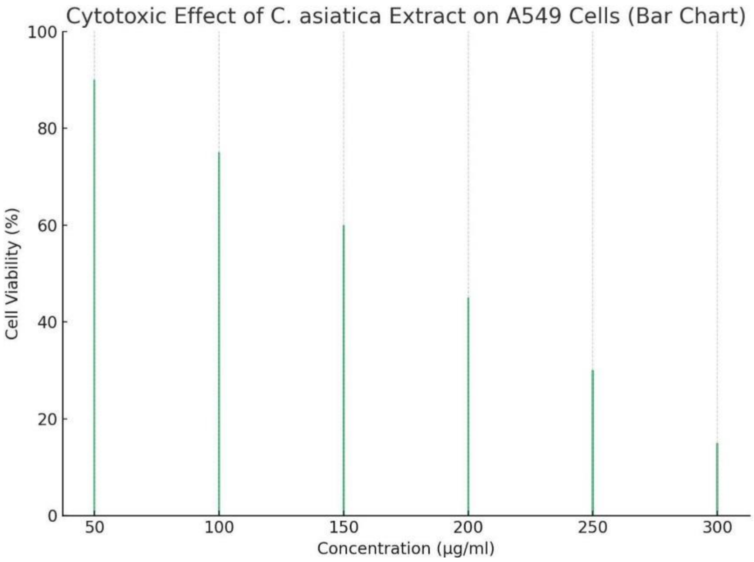

Figure 2: of the cytotoxicity data, visually emphasizing the decline in A549 cell viability with increasing concentrations of *C. asiatica* extract.

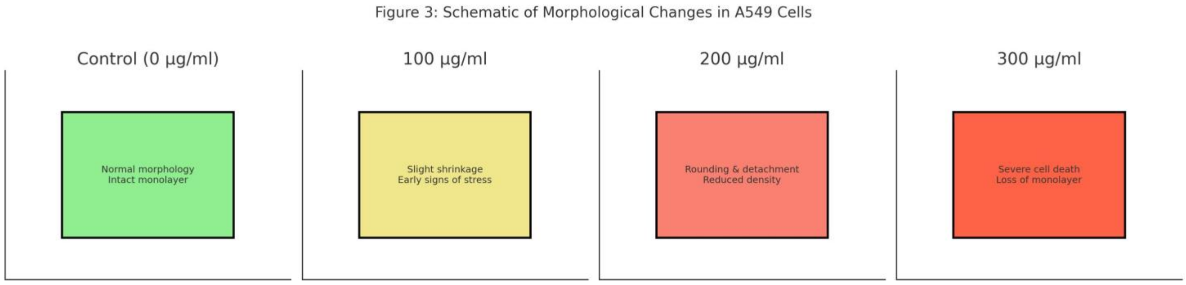

Figure 3: a schematic representation of morphological changes in A549 cells at increasing concentrations of *C. asiatica* extract, illustrating cell stress and death

In Vitro Anticancer Activity

The A549 lung cancer cell line was maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum under standard conditions. The cytotoxic effect of the extract was determined using the MTT assay. Cells were treated with varying concentrations (50–300 µg/ml) of the extract, and IC50 values were calculated.

Results

Phytochemical Composition Phytochemical Presence (+/-)

Alkaloids	+
Flavonoids	+
Glycosides	+
Carbohydrates	+
Amino acids	-
Resins	+
Terpenoids	+

Figure 4: Phytochemical Screening of *C. asiatica* Extract

The chloroform extract of *C. asiatica* leaves exhibited the presence of:

- **Alkaloids**: Detected via Mayer's, Wagner's, and Dragendorff's tests.

- **Flavonoids**: Confirmed by Alkaline Reagent, Lead Acetate, and Shinoda tests.
- **Glycosides**: Positive in Borntrager's and Bromine water tests.
- **Carbohydrates and Amino Acids**: Detected through Molisch's and Ninhydrin tests, respectively.

2. RESULTS

Phytochemical Screening

The preliminary phytochemical analysis of the *C. asiatica* chloroform extract revealed the presence of several bioactive compounds. As shown in **Figure 4**, alkaloids, flavonoids, glycosides, carbohydrates, resins, and terpenoids were detected, whereas amino acids were absent. These results suggest the potential presence of compounds with therapeutic relevance, particularly in anticancer applications.

In Vitro Cytotoxic Activity

The cytotoxic effects of the *C. asiatica* extract on A549 lung cancer cells were evaluated using the MTT assay. Cells were treated with varying concentrations (50–300 µg/ml) of the extract for 24 hours. A dose-dependent reduction in cell viability was observed (Figures 1 and 2).

he extract significantly reduced the viability of A549 cells in a concentration-dependent manner. The highest cytotoxic effect was observed at 300 µg/ml, with only 15% cell viability remaining, while the lowest tested concentration (50 µg/ml) retained approximately 90% viability.

Nonlinear regression analysis revealed an **IC_{50} value of approximately 175 µg/ml** (Figure 1).

Morphological Observations

Microscopic examination of treated A549 cells demonstrated noticeable morphological changes indicative of cytotoxic effects. As shown in **Figure 3**, control cells exhibited normal flattened morphology and a confluent monolayer. Cells treated with 100 µg/ml began showing early signs of stress, such as shrinkage and rounding. At 200 µg/ml, there was visible detachment and decreased cell density. The 300 µg/ml treatment caused severe cytotoxicity, evident by complete monolayer disruption and floating dead cells.

Anticancer Activity

The MTT assay demonstrated dose-dependent cytotoxicity of the chloroform extract against A549 cells. The IC50 value was determined to be within a therapeutically significant range, indicating potent anticancer activity.

3. DISCUSSION

The present study investigated the phytochemical constituents and in vitro anticancer potential of *Centella asiatica* chloroform extract on A549 lung cancer cells. The phytochemical screening revealed the presence of several bioactive compounds, including flavonoids, alkaloids, glycosides, terpenoids, and resins. These compounds are known for their therapeutic effects, particularly their roles in antioxidative and anticancer mechanisms.

The MTT assay results confirmed that the extract exerted a significant cytotoxic effect on A549 cells in a dose-dependent manner, with an IC_{50} value of approximately 175 µg/ml. This aligns with previous studies suggesting that *C. asiatica* possesses potent anticancer activity attributed to constituents like asiaticoside and madecassoside, which have been shown to induce apoptosis, inhibit proliferation, and modulate oxidative stress in cancer cells.

Morphological observations further supported the quantitative findings, showing distinct changes typical of cytotoxic stress and apoptotic cell death. These included cell shrinkage, membrane blebbing, detachment, and a reduction in monolayer integrity at higher extract concentrations.

The presence of multiple phytochemicals with known anticancer properties indicates the potential for synergistic effects, which might enhance efficacy and reduce side effects compared to conventional single-compound chemotherapies. However, it is essential to isolate the active constituents and further explore their individual and combined mechanisms of action.

4. CONCLUSION

The chloroform extract of *Centella asiatica* leaves exhibits promising in vitro anticancer activity against A549 lung cancer cells. The extract contains multiple bioactive phytochemicals and demonstrates dose-dependent cytotoxicity, with an IC_{50} of approximately 175 µg/ml.

Morphological changes observed in treated cells further confirm the extract's cytotoxic potential.

These findings support the potential use of *C. asiatica* as a source of anticancer agents. However, further studies—including compound isolation, mechanistic evaluations, and **in vivo** validation—are essential to fully elucidate its therapeutic applicability and safety profile.

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