Apoptosis Induction and S-Phase Cell Cycle Blockade in Human Lung Adenocarcinoma Cell Line (A549) by *Chlorophytum comosum* (Thunb.) Jaques

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Research Article

Keywords: Apoptosis, A549, Chlorophytum comosum, Lung Cancer, Spider Plant, Phytoterapeutics

Posted Date: January 29th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-5897484/v1

Additional Declarations: The authors declare no competing interests.

Abstract

Methods

The ethanolic roots (CCRE) and leaves (CCLE) extracts were interrogated for their apoptotic potential against human lung adenocarcinoma cell line (A549) using DNA fragmentation, Annexin V-FITC/PI staining apoptosis assay and cell cycle analysis using flow cytometry.

Results

Our results revealed significant DNA damage and apoptosis induced cell death in A549 cell line on treatment with active concentrations (40 µg/ml and 80 µg/ml) of the ethanolic extracts with S phase cell cycle arrest.

Conclusions

This is the first study demonstrating the apoptosis inducing potential of chemically characterized bioactive compounds present in ethanolic leaves and roots extracts from *Chlorophytum comosum* against non-small cell human lung adenocarcinoma cell line. The study concludes that *Chlorophytum comosum* can be a potential candidate for the natural bioactive compounds that can be isolated, characterized and clinically evaluated for the development of novel naturally derived anti-cancer drugs against lung cancer.

1. Introduction

Lung cancer is the second most aggressive type of cancer contributing highest (21%) of all cancer deaths [1]. Unlike other cancer types, where often the risk of cancer occurrence is influenced by genetic and epigenetic factors; lung cancer in majority, is a detrimental consequence of smoking habits observed among the human population and continued direct exposure of the harmful carcinogens present in the environment. Lung cancer incidence and mortality rates are tightly linked to cigarette smoking patterns [2]. Another challenge associated with lung cancer is its delayed detection mostly at advanced stage. Approximately 44% of people have advanced stage disease at diagnosis [2]. According to American Cancer Society, in US alone, approximately 101,300 (81%) of the 125,070 lung cancer deaths in 2024 will be caused by direct cigarette smoking with an additional mortality of 3500 which will be caused by second hand smoke [1]. Despite the availability of modern chemotherapeutic interventions like personalized immunotherapy, chemotherapy, radiotherapy and surgical methods, the 5-year relative survival rate for all stages combined in lung cancer is 25% [1]. The side effects of lung cancer treatment by these approaches render patients to look for alternative therapies that may help in improving the quality of life and naturally delaying the recurrent episodes of severe side effects as the disease progresses. Several forms of complementary and alternative medicine practiced under different

traditional medicinal systems are now under investigation for its potential role in curbing and combating cancer [3, 4]. In China, Traditional Chinese Medicine (TCM) has been widely accepted as a mainstream form of complementary and alternative therapy for cancer patients [5, 6, 7]. A large number of studies on Traditional Chinese Medicine are elucidating the role of the herbs at the molecular level to understand its impact on cancer signaling pathways [8, 9, 10].

Chlorophytum comosum (Thunb.) Jaques belongs to family Lilliaceae and genus *Chlorophytum* [11]. It is commonly known as Spider plant and is worldwide popular for its ornamental value. In Traditional Chinese Medicine, it is used for the treatment of fractures, burns and respiratory ailments such as bronchitis and asthma [12, 13]. In India, roots of *Chlorophytum comosum* are used as a substitute for another medicinal *Chlorophytum* species (*Chlorophytum borivilianum*) for preparation of an important class of ayurvedic drugs known as Rasayna [14]. Besides, several other species belonging to genus *Chlorophytum* are well known for their pharmacological properties [15, 16]. The genus *Chlorophytum* is predominantly rich in saponins and has been extensively studied for its cytotoxic properties [17]. However, as far as *Chlorophytum comosum* is concerned not much work has been done to discover its potential as an anticancer herb. Preliminary findings from earlier studies indicates its cytotoxic potential against selected cancer cell lines, however, these studies were only limited to initial cytotoxicity screening without deciphering the mechanism of action [18, 19]. Saponins isolated from roots of *Chlorophytum comosum* have been found to exhibit cytotoxic and anti-tumour promoter activity in HeLa cancer cell line [18]. In an another important study, DNA damage in four different human cancer cell lines viz. HeLa, CCRF-HSB-2, HL-60 and U937 cells under the effects of n-butanol root fraction from *Chlorophytum comosum* was demonstrated using qualitative DNA fragmentation assay and the Terminal Deoxynucleotidyl Transferase (TdT) mediated biotin dUTP Nick End Labeling (TUNEL) method [19]. In a study, methanolic leaf extract of *Chlorophytum comosum* was evaluated for its chemical profile and its different sub fractions were explored for the bioactivity. The study revealed steroids and isoprenoid as the most abundant compounds present in methanolic leaf extract with higher percentage of unsaturated fatty acids and neophytadiene as the second highest abundant compound. Biological assessment of its sub fractions viz. chloroform, n-hexane, n-butanol and water revealed significant *in vitro* antioxidant and selective cytotoxic activity against HeLa cancer cell lines [20]. Recently, progressive neuroprotective efficacy of roots and leaves extracts of *Chlorophytum comosum* at the concentrations of 60 µg/ml and 90 µg/ml were demonstrated against glutamate excitotoxicity in a culture of rat cerebellar neurons retrieved from 7–9 day old rats [21]. *Chlorophytum comosum* inspite of having a strong Ethnopharmacological base and as an important ingredient in nutritional/health supplement in different traditional medicinal systems such as Traditional Chinese Medicine (TCM), African Traditional Medicine (ATM) and Ayurveda is yet unexplored on scientific grounds for its therapeutic potential [12, 13, 22, 23, 14]. Moreover, with view of its traditional usage for treatment of respiratory ailments, so far, no biological activity has been reported to explore its antiproliferative efficacy against lung cancer. Thus, keeping in view of the earlier literature and Ethanomedical lead, the present study aimed to further investigate the anticancer potential of ethanolic roots and leaves extract of *Chlorophytum comosum* against human

lung adenocarcinoma cell line (A549) by understanding the mode of cancer cell death prompted by the extracts.

2. Materials and Methods

2.1 Chemicals and Reagents

Solvents used in the study i.e. n-Butanol, Petroleum Ether, Water, were of HPLC/LC-MS grade and were purchased from Merck (Darmstadt, Germany). Ethanol (China grade) and Hydrogen peroxide solution was purchased from local commercial supply (Thomas Baker, India). Cell culture media DMEM with phenol red (#1932403), RPMI-1640 (# 1898961), Fetal Bovine Serum (#10438034), Trypsin–EDTA solution with phenol red (#1897336) and antibiotic solution PenStrep (#192493) were purchased from Gibco USA. MTT reagent (#MICB8173V), Propidium iodide (# P4864), RNase A, Standard Doxorubicin and Colchicine were obtained from Sigma Aldrich USA. FITC-Annexin V apoptosis detection kit was obtained from Invitrogen USA (# V-13242). Standard Vinblastine sulphate was purchased from Cipla (India). Sheath buffer was purchased from BD Biosciences-IN. All the cell lines used in the study were procured from ATCC (USA).

2.2 Plant Material Collection

Mature roots and leaves part of *Chlorophytum comosum*; cultivated for a period of 180 days were harvested in June 2016 from Herbal Garden, Jamia Hamdard, New Delhi. The plant parts collected were healthy and disease free. The plant samples were identified by the botanist Dr. Sunita Garg (NISCAIR, New Delhi) and the voucher specimens bearing no. NISCAIR/RHMD/CONSULT/ 2016/2975-02 were deposited in the herbarium.

2.3 Drug Preparation and Treatment for MTT Assay

For MTT assay, the semi dried extracts were weighed and initially prepared in DMSO with a final concentration of DMSO < 0.1%. The samples were two fold serially diluted to 6 different concentrations (10–320 µg/ml) [Stock conc. 3.2 mg/ml; Working conc. 0.32 mg/ml] using incomplete DMEM medium. Vinblastine sulphate served as positive control with 6 different test concentrations (3.12–100 µM) [Stock conc.1.2 mM] [24]. Cells with 1% DMSO served as vehicle control, while, cells with only media served as negative control. Time duration for treatment was 24 hrs.

2.4 Cell Lines Maintenance

Human lung adenocarcinoma cell line (A549) [CCL-185] and human normal lung cell line (L-132) [CCL-5] were procured from the American Type Culture Collection (Manassas, VA, USA). The cell lines obtained were pre- authenticated (molecularly characterized). Cell lines quality control specifications and authentication documentation were confirmed from the available information on the ATCC website as reference. Cell lines were maintained in DMEM/RPMI-1640 medium supplemented with 10% heat inactivated FBS and 1% penicillin/streptomycin solution with 5% CO_2 supply at 37° C.

2.5 Experimental

2.5.1 Preparation of Plant Extracts

Ethanolic extracts of roots (CCRE) and leaves (CCLE) of *Chlorophytum comosum* were prepared using hot continuous Soxhlet extraction method. Briefly, fresh plant materials were collected and washed to remove debris, shade dried under sunlight for seven consecutive days to remove the moisture content until no change in weight was observed. The grinded plant material (10 gm) each was defatted using petroleum ether (1:15 w/v), dried and suspended in ethanol (150 mL), and extracted using Soxhlet apparatus for 24 hrs at 50^0 C. The extracts were then collected, filtered, and partitioned using n-butanol until the upper layer became colorless. The upper layer was further collected, pooled and concentrated on a rotary evaporator at 55^0 C and 55 mbar pressure until the semi-dried substance was obtained. The semi-dried substance was further lyophilized to remove the traces of the solvent. The samples were then stored in airtight vials at 4^0 C till further use.

2.5.2 Cell Cytotoxicity Assay by MTT

Representing methodology from our previous data for continuation of the study [25], the cytotoxic activity in terms of % cell inhibition by roots ethanolic (CCRE) and leaves ethanolic (CCLE) extracts were measured using cell based colorimetric (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction MTT assay with the treatment time of 24 hrs [26]. Briefly, monolayer cell culture (A549 & L-132) grown in T_{25} cell culture flask was trypsanized using trypsin EDTA solution with phenol red and collected as pellet after centrifugation at 1000 rpm in 1 ml of complete medium. The total number of viable cells per ml was then counted with the help of Trypan blue dye (1:1 ratio with dilution factor 2) using hemocytometer. The cell count was adjusted to 40,000 cells/well, and to each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension containing 40,000 cells was added. After 24 hrs, media was removed, cells were washed once with fresh medium and further treated with 100 µl of varying concentrations of extracts (10–320 µg/ml) and Vinblastine (3.12–100 µM) for 24 hrs. Subsequently, post treatment, 10 µL of MTT dye (0.5 mg/mL) was loaded inside each well and incubated further for 4 hrs at 37^0 C in an incubator supplied with 5% CO_2. MTT solution was removed and crystals formed were then dissolved in DMSO. Finally, absorbance was taken at 570 nm using microplate reader (Spectra Max, USA). The % cell inhibition was measured by using the following formula (Eq A.1). The concentration at which the test drug inhibited cell growth by 50% i.e. inhibitory concentration (IC_{50}) was generated from the dose-response curves by nonlinear regression analysis using Graph Pad Prism software (8.1).

% Cell Inhibition by CCLE /CCRE extracts = [(C – S) X 100] / C ……. (Eq A.1)

C = Absorbance of control; S = Absorbance of samples.

2.5.3 Determination of Test Doses Based on IC_{50} Values

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process like cell growth by half. To determine the apoptosis inducing effects of *Chlorophytum comosum* leaves ethanolic extract (CCLE) and roots ethanolic extract (CCRE), the active lower and higher doses were set corresponding to the determined IC_{50} values of each extract from our previously published data [25]. The doses ranges selected for CCRE with an IC_{50} value of 68.68 µg/ml and CCLE with an IC_{50} value of 61.19 µg/ml were 40 µg/ml & 80 µg/ml respectively. Both the IC_{50} values were falling between these two upper and lower doses selected for the study.

2.5.4 DNA Fragmentation Assay

Qualitative estimation of apoptosis inducing effects of the *Chlorophytum comosum* ethanolic extracts were measured using DNA fragmentation ladder assay. The formation of distinct DNA fragments of oligonucleosomal size (180–200 bp length) is a biochemical hallmark of apoptosis in many cells [27]. These DNA fragments can be extracted from the cells post treatment and visualized using agarose gel electrophoresis method. Briefly, A549 cells seeded in DMEM medium at a concentration of 8 x 10^5 cells per 35 mm dish, incubated at 37^o C, 5% CO_2 were treated with two concentrations i.e. (40 µg/ml and 80 µg/ml) of each sample (CCRE & CCLE) for 24 hrs. Hydrogen peroxide served as control [28]. Total genomic DNA was taken out and DNA damage was evaluated using 1% agarose gel [29]. DNA ladder of 1500 bp served as marker ladder to determine the apoptotic fragments.

2.5.5 Cell Cycle Analysis by Propidium Iodide

Cell cycle analysis was performed by PI (Propidium iodide) staining method using flow cytometry [30, 31]. Briefly, A549 cells (8 x 10^5 cells/ml) cultured using 2 ml of DMEM medium in 6 well plates were treated with desired concentrations (40 µg/ml and 80 µg/ml) of test extracts (CCLE & CCRE) for 24 hrs. Post incubation, the medium was discarded and cells were gently scrapped using BD cell scrapper, transferred to RIA vials and centrifuged at 4500 rpm for 5 min at 4^0C. The supernatant was discarded carefully retaining the cell pellets. Cell pellets were further washed twice using 1X PBS and fixed by resuspending in 300 µl of Sheath fluid followed by addition of 1mL of chilled 70% ethyl alcohol drop by drop with continuous gentle shaking and another 1 mL of chilled 70% ethyl alcohol added at once. The samples were stored at 4^0 C. Subsequently, cell pellets were washed twice with 2 ml of ice cold 1X PBS and then suspended in 450 µl of sheath fluid containing 16 µl PI [Stock conc. 0.05 mg/ml] and 16 µl RNaseA [Stock conc. 2 mg/ml; Working conc. 1 mg/ml] and incubated for 30 min in dark. The percent DNA content in various cycle phases in treated versus negative (untreated) control cells were determined using FACS Caliber instrument (BD Biosciences) with Cell Quest software (BD Biosciences, San Jose, CA, USA). Colchicine treated A549 cells (25 µM) served as positive control [32].

2.5.6 Apoptosis Detection Using PI/Annexin V-FITC Staining

Apoptosis detection was performed using a commercial kit (Invitrogen V-3242) following the manufacturer's recommendations. Briefly, 8 x 10^5 A549 cells/ml plated in DMEM medium were treated with 40 and 80 µg/ml of CCLE and CCRE extracts for 24 hrs. The cells were then harvested, centrifuged at 4500 rpm for 5 min at 4^0 C and washed twice with ice-cold 1X PBS. The pellets were suspended with the 1X binding buffer (100 µL) and then PI (10 µL) [Stock conc. 1mg/ml] and Annexin V-FITC (3 µL) was added. The suspension was incubated in dark for 10 min at room temperature (RT). Before flow cytometry analysis, 100µL of 1X binding buffer was further added to the suspension. Data from 10,000 cells per sample were collected and analyzed by flow cytometry using a FACS Caliber instrument with Cell Quest software (BD Biosciences, San Jose, CA, USA). The results were compared with the untreated negative control A549 cells. Doxorubicin treated A549 cells (25 µM) served as positive control [33].

2.5.7 Statistical Analysis

The statistical analysis was performed with one way analysis of variance (ANOVA) to calculate the statistical significance with $p < 0.05$ as considered significant. The inhibitory concentration (IC_{50}) values were calculated by nonlinear regression curve (log inhibitor vs. normalized response variable slope) with the use of Graph pad Prism version 8.1 for Windows (Graph pad Prism Software, San Diego, CA, USA). All results were expressed as mean ± standard deviation.

3. Results

3.1 Selective Cytotoxicity of *Chlorophytum comosum* against Human Lung Adenocarcinoma (A549) Cell Line by MTT Assay

Based on our earlier published data [25] on antiproliferative effects of *Chlorophytum comosum*, dose dependent selective cytotoxic activity towards A549 lung cancer cell line with an IC_{50} values of 68.68 µg/mL and 61.19 µg/mL were demonstrated by both roots ethanolic (CCRE) and leaves ethanolic (CCLE) extracts respectively **(Table 1)**. Leaves extract showed better cytotoxic response as compared to roots extract with lesser IC_{50} values. In positive control taken as Vinblastine sulphate, maximum inhibitory effect with an IC_{50} value of 18.79 µM and 20.71 µM was observed in A549 and L-132 respectively [25]. While in case of normal human lung cell line (L-132), the IC_{50} values were not calculated due to lesser percentage inhibition i.e.< 50%, indicating non-significant activity. For the present study, the test doses were selected as two active concentrations i.e. 40 µg/ml and 80 µg/ml for both roots ethanolic and leaves ethanolic extracts representing the lower and upper ranges between the determined IC_{50} values to carry out the further investigations.

3.2 Chlorophytum comosum Leaves Ethanolic (CCLE) and Roots Ethanolic (CCRE) Extracts Promoted Nuclear DNA Fragmentation in A549 Cell Line

One major characteristic feature of apoptotic mode of cell death is the extensive morphological and biochemical changes that ensure the vanishment of dying cells without affecting and harming the other neighboring cells. In contrast, during necrosis mode of cell death, the cell gets destroyed leaving the traces of cellular contents that activate the chain reaction of inflammation in the cellular microenvironment. Formation of short distinct fragments of DNA of 180–200 bp length visualized on agarose gel represents DNA damage and is considered as a biochemical hallmark of apoptosis process [27]. The apoptosis induction in A549 human lung adenocarcinoma cell line by the roots ethanolic and leaves ethanolic extracts of *Chlorophytum comosum* was qualitatively reported using gel based DNA fragmentation assay. A dose dependent internucleosomal fragmentation pattern was observed in A549 cells on 1.8% agarose gel stained with ethidium bromide when treated with CCLE extract at the concentrations of 40 µg/ml and 80 µg/ml for 24 hrs. Fine smearing throughout the gel represented significant DNA damage. Similarly in case of CCRE extract, significant DNA damage was observed at both doses, however, compared to leaves (CCLE) extract, the damage in roots extract was moderately recorded at 80 µg/ml. Hydrogen peroxide taken as positive control was used to compare the fragmentation pattern. Overall, these results suggested that both extracts induced DNA fragmentation in A549 cell line indicating DNA damage by apoptosis **Fig. 1 (a & b).**

3.3 *Chlorophytum comosum* Induced Early and Late Apoptotic Cell Death in A549 Cells

The apoptotic effect of *Chlorophytum comosum* roots ethanolic (CCRE) and leaves ethanolic (CCLE) extracts was confirmed using Annexin- FITC/PI staining by evaluating the stages of cell death being early apoptotic, late apoptotic and necrotic. Apoptosis initiation is markedly represented by the acquisition of surface changes by dying cells such as the expression of thrombospondin binding sites, loss of sialic acid residues and exposure of phospholipids like phosphatidylserine (PS) [34]. Early apoptotic events start with the translocation and exposure of phosphatidylserine to the outer layer of the membrane with intact cell membrane. True apoptotic cells can be quantitated using Annexin V dye which preferentially binds to negatively charged phospholipids like PS in the presence of Ca^{2+}; the events of which are recorded in the form of fluorescence signal by flow cytometry. Staining cells simultaneously with FITC-Annexin V and the Propidium iodide allows the discrimination of intact cells by early apoptotic, late apoptotic or necrotic cells [34]. Apoptosis induction studies using Annexin V-FITC/PI staining demonstrated the occurrence of apoptosis in A549 cells after treatment with CCLE and CCRE extracts for 24 hrs. Dose dependent decrease in cell viability with varying % of cells in early apoptotic, late apoptotic and necrosis stages were recorded **Fig. 2a.** The 40 and 80 µg/ml treatment of leaves extract (CCLE) induced early apoptosis by 5.69%, 1.95%, and late apoptosis by 10.19%, 7.21% respectively. The roots extract (CCRE) treatment at 40 µg/ml and 80 µg/ml has induced early apoptosis by 9.67%, 5.38% and late apoptosis by 3.98%, 8.15% respectively. Necrotic cells were found to be 13.71%, 30.32% and 4.77%, 16.93% at 40 µg/ml and 80 µg/ml in CCLE and CCRE extracts respectively **Fig. 2b.** In control untreated A549 cells, 91% of viable cell remained intact with 8.32% early apoptotic cells with negligible late apoptotic cells i.e. 0.21% and no necrotic detected cells **Fig. 2b.** These results indicate that the death of A549 cells induced by *Chlorophytum comosum* was mediated by apoptosis. Doxorubicin used

as positive control (standard), detected 14.44% early apoptotic, 30.66% late apoptotic, and 12.21% necrotic cells with 42.69% of viable cells **Fig. 2b.**

3.4 *Chlorophytum comosum* Arrested Cell Cycle Progression at S Phase

The effects of leaves ethanolic (CCLE) and roots ethanolic (CCRE) extracts of *Chlorophytum comosum* were seen on cell cycle progression analyzed using PI staining detected by flow cytometry method. The treatment of A549 cells with CCLE and CCRE extracts for 24 hr at two different dosages (40 µg/ml and 80 µg/ml) led to the cell cycle arrest at S phase **Fig. 3a.** The cell cycle arrest was found to be in dose dependent manner with two fold change which was observed in both the cases from lower to higher concentrations, however, the leaves extract was found to be most effective in accumulating and arresting cells at S phase followed by the roots extract. CCLE extract has shown dose dependent S phase arrest of 23.23% and 42.86% at 40 µg/ml and 80 µg/ml respectively, compared to control untreated cells which showed only 5.90% arrest in A549 cells at S phase. While in CCRE extract, S phase arrest was 19.64% at 40 µg/ml and 38.03% at 80 µg/ml. Standard Colchicine at 25 µM showed 51.33% S phase arrest and 5.08% G2 arrest in A549 cells **Fig. 3b.**

4. Discussion

The exploration of anticancer drugs from plant source is rapidly evolving with various high ends *in vitro* and *in silico* screening techniques which helps in the preliminary identification of the active target biomolecules that may serve as lead candidates in anticancer therapy [35, 36]. Several phytochemicals from plants have been found to possess anticancer activity by virtue of their ability in modulating the cell growth regulatory mechanisms i.e. cell cycle regulation and by inducing apoptosis in cancer cells when treated [37, 38]. The apoptosis inducing ability of the phytoconstituents in immortal cancer cells is thus, a remarkable attempt to control the progression and spread of the cancer. This is achieved by the manipulation and activation of various cancer signaling pathways at the molecular level [36, 39]. In the present study, an attempt has been made to evaluate the anticancer potential of ethanolic roots and leaves extracts from *Chlorophytum comosum* against A549 human non-small cell lung carcinoma cell line by determining the mode of cell death and its underlying mechanism. *Chlorophytum comosum* is reported to be used in treatment of various respiratory ailments in Traditional Chinese Medicine (TCM) and has been found to be useful in relief from bronchitis and asthma [12, 13]. Saponins are the chief active constituents found in *Chlorophytum comosum* and possess cytotoxic potential against cancer cell lines [17–19]. However, till date no scientific studies were carried out with regard to respiratory ailments that could also justify its ethnomedical importance. To our best of knowledge, our study for the first time evaluated the apoptosis inducing potential of *Chlorophytum comosum* against lung cancer using *in vitro* human lung adenocarcinoma cell line (A549) model which is an extensively studied model for human lung epithelial cancer and possesses the high metastatic potential [40]. This work is in continuation of our previously published work, where cytotoxicity studies by MTT assay revealed significant antiproliferative effects by chemically characterized ethanolic roots and leaves extracts

selectively towards A549 cell line. While, no antiproliferative effects of the extracts at the same tested concentrations were observed against normal human lung cell line L-132. On the other hand positive control Vinblastine exhibited significant cytotoxicity towards both A549 and L-132 cell lines. In the present study, based on the earlier demonstrated IC_{50} values, two active concentrations of the extracts were selected and we further investigated the markers of cell death process to ascertain the bioactivity of *Chlorophytum comosum.* We observed a systematic mode of cell death via apoptosis in A549 cells under the effect of the treatments. The early markers in apoptosis induced cell death involve internucleosomal DNA damage, cell membrane disruption and expression of Phosphatidylserine on outer membrane surface [34]. In our study, all these changes were systematically evident in A549 lung cancer cells post treatment. Annexin V-FITC/PI flow cytometry studies revealed increase in early apoptosis, late apoptosis and necrotic cells after CCLE and CCRE extracts treatment for 24 hrs with dose dependent decrease in the cell viability. DNA fragmentation studies indicated the DNA damage initiated by the treatment in response to non-treated control cells where no such activity was observed. However, the potency of each extract exhibited varied response at different concentrations selected for the study. CCLE extract exhibited greater potential as compared to CCRE extract. Another aspect associated with cancer control and progression is cell stage specific growth during which many proteins are altered and leads to malignant conditions. Studies reveals that plant derived drugs block cell cycle progression at various stages of cell cycle such as G0/G1, S or G2/M phases to induce apoptotic cell death [40, 41, 42]. Our study reveals the arrest of cell cycle of malignant A549 cells when treated with CCLE and CCRE extracts at S phase **Fig. 3**. The growth arrest and apoptosis inducing features of the plant extracts have been found significant in number of studies. In a study conducted by Yerlikaya et al. [43], ethyl acetate extract from *Lotus corniculatus* exhibited significant apoptotic driven cell death via increased intranuclear influx of $Ca2^{+}$ in MDA-MBA-231 breast cancer cell line. In a similar findings by Lee et al. [44], ethanolic extract from *Calotropis gigantean* induced apoptosis through the activation of extrinsic and intrinsic pathways, cell cycle arrest, and ROS generation in A549 and NCI-H1299 lung cancer cell lines. In a study conducted by Samarghandian et al. [45], antineoplastic potential of a plant derived Thymoquinone was assessed using flow cytometry studies on A549 cell line and were found to significantly up regulate the apoptotic modulators viz. *p53*, caspase 3 and 9 with significantly elevated Bax/Bcl-2 ratio thereby, concluding initiation of apoptosis in A549 cells via caspase cascade dependent pathways. Thus, with insights from earlier literature and following the lead of our previous work where significant cytotoxic effects of ethanolic extracts of *Chlorophytum comosum* were evident on human lung carcinoma cell lines; the present study confirms the antiproliferative and antitumor effects of CCLE and CCRE extracts in A549 lung cancer cell line. This the first report of lung cancer cell toxicity and programmed cell death via apoptosis pathway in A549 cell line by *Chlorophytum comosum* ethanolic roots and leaves extracts. Such data indicates that *Chlorophytum comosum* have therapeutic phytochemical leads as apoptosis inducers in lung cancer cells that can be further isolated, characterized and studied and their potency as anticancer agents could be explored in the management of lung cancer. However, persuasive investigation is required to further characterize and elucidate the cancer signaling pathways involved in the bioactivity of active constituents from *Chlorophytum comosum*.

5. Conclusions

Active constituents from roots and leaves parts of *Chlorophytum comosum* possess significant cytotoxic and apoptosis inducing potential against A549 lung cancer cell line suggestive of its anticancer potential. However, further in line research, based on *in vivo* and clinical studies will reveal novel bioactive leads from *Chlorophytum comosum* (Thunb.) Jaques.

Abbreviations

CCRE
Chlorophytum comosum roots ethanolic extract
CCLE
Chlorophytum comosum leaves ethanolic extract
FBS
Fetal Bovine Serum
PBS
Phosphate Buffer Saline
A549
Human Lung Adenocarcinoma Cell Line

Declarations

Financial Support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data Availability Statement

The raw data that support the findings of this study are available from the corresponding author [Dr. Humaira Farooqi] upon reasonable request.

Acknowledgements

The authors are thankful to Professor M.Z. Abdin (Department of Biotechnology, Jamia Hamdard) for providing facilities for sample preparations. Mr. Hashmat (Herbal Garden, Jamia Hamdard) for supervising and providing plant samples. Dr. Sunita Garg and Professor M.P. Sharma for authentication of plant samples and Hamdard National Foundation for granting research fellowship to Dr. Adhami. The kind guidance and technical assistance of Skanda Life Sciences, Bangalore is highly appreciated.

Declaration of Interest

The authors report there are no competing interests to declare.

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Figures

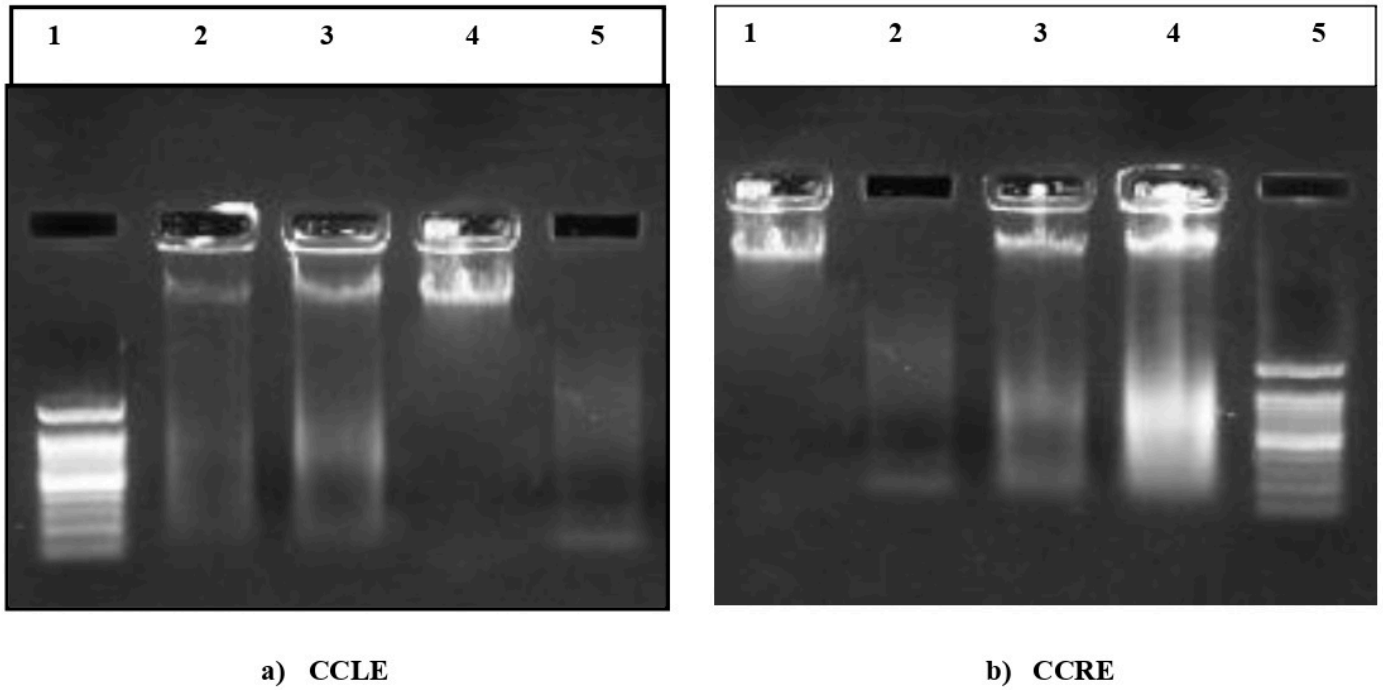

Figure 1

(a & b). DNA Fragmentation Analysis Induced by Ethanolic Leaves (CCLE) and Roots (CCRE) Extracts from *Chlorophytum comosum* in A549 Cells. Induction of DNA fragmentation in A549 cells observed post CCLE and CCRE treatment. A total of 8 x 105 cells were exposed at (40 µg/ml and 80 µg/ml) for 24 hrs. DNA was electrophoresed on 1.8% agarose gel and stained with ethidium bromide. **Fig 1(a)** CCLE induced dose dependent DNA fragmentation pattern in A549 cell line. **Lane 1**- DNA marker ladder (1500 bp), **Lane 2**- 80µg/ml, **Lane 3**- 40 µg/ml, **Lane 4**- negative control (untreated A549 cells), **Lane 5**- positive control H2O2 **, Fig. 1(b)** CCRE treated cell line showing moderate DNA fragmentation pattern. **Lane 1**- negative control (untreated A549 cells), **Lane 2**- positive control H2O2, **Lane 3**- 40 µg/ml, **Lane 4**- 80µg/ml, **Lane 5**- DNA marker ladder (1500 bp).

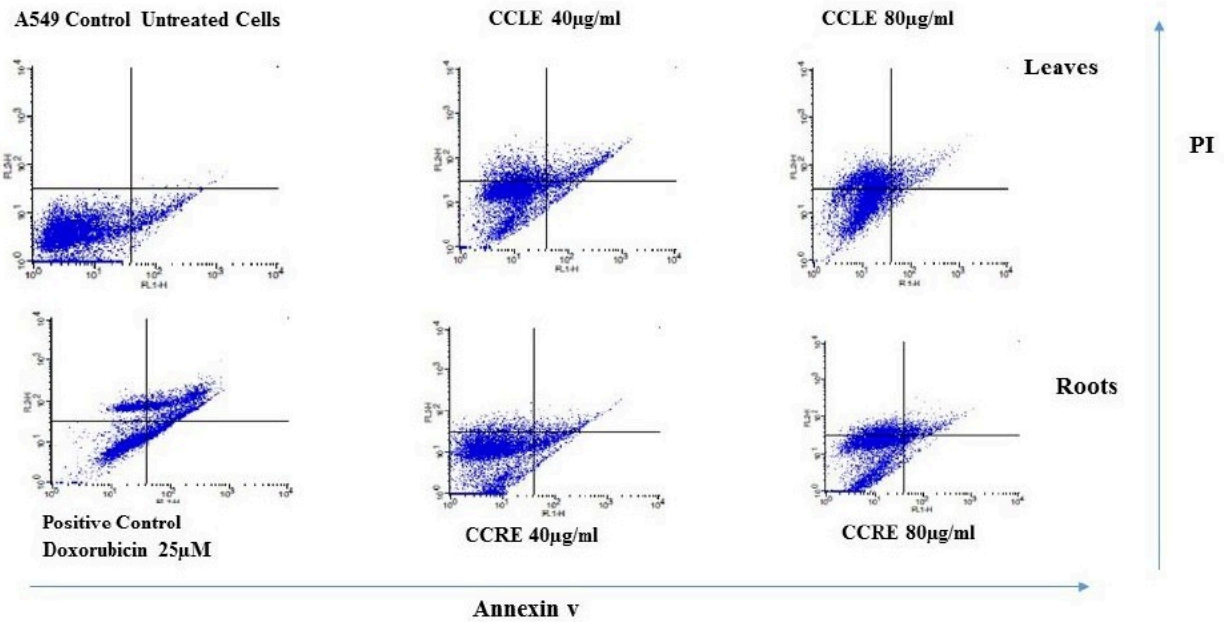

Figure 2a

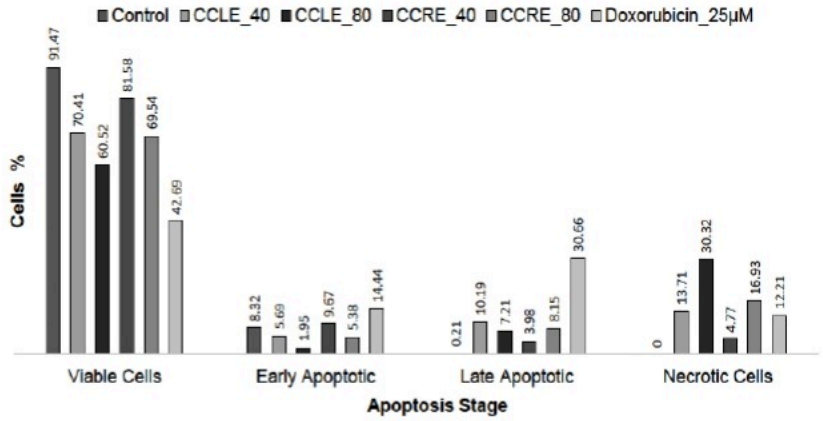

Figure 2b

Figure 2

a Apoptosis Detection in Ethanolic Leaves (CCLE) and Roots (CCRE) Extracts Treated A549 Cells using PI-Annexin-V/FITC Staining by Flow Cytometry. Apoptosis induction was confirmed in A549 cell line by *Chlorophytum comosum* roots (CCRE) and leaves (CCLE) extracts after 24 hrs of exposure using Annexin V-FITC/PI staining. Flow cytometry analysis representing four quadrants indicating different stages of apoptosis. **Upper Left (UL)**- Dead Necrotic Cells, **Upper Right [A+/PI+]**- Late Apoptotic Cells, **Lower Left (LL)**- Viable Cells **[A-/PI-], Lower Right (LR) [A+/PI-]**-Early Apoptotic Cells.

b FACS Analysis of Apoptosis Detection in A549 Cells. Bar diagram representing percent apoptosis at different stages with dose dependent decrease in cell viability. Data is statistically significant at $p < 0.05$.

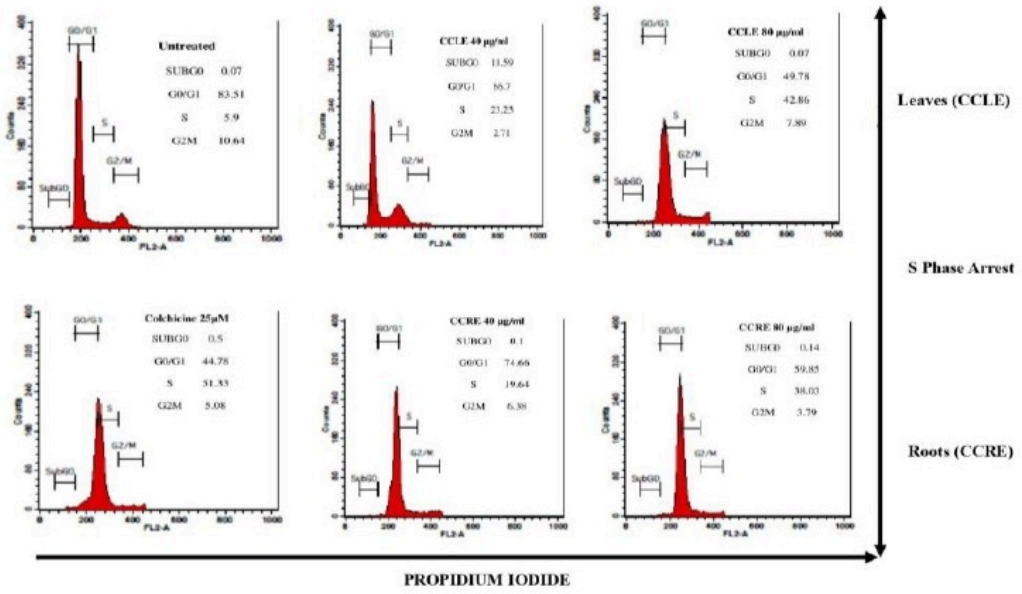

Figure 3a

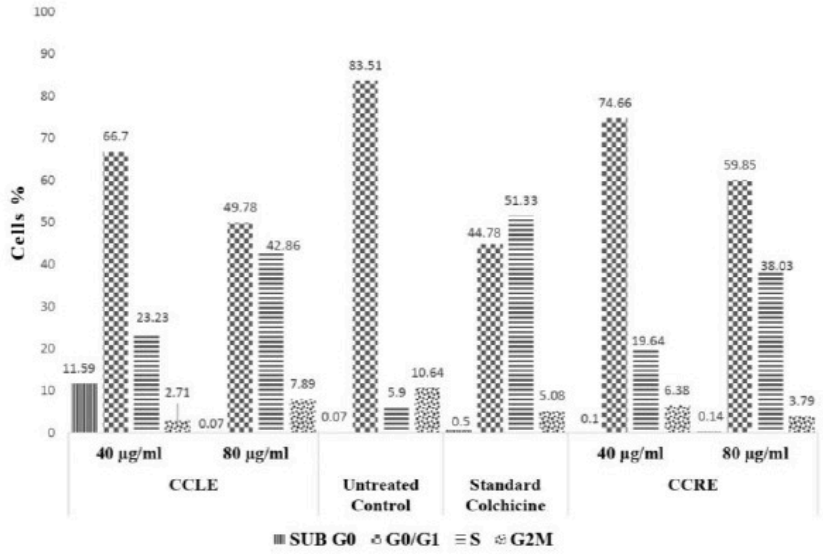

Figure 3b

Figure 3

a. Cell Cycle Progression Analysis of Treated A549 versus Untreated A549 Cancer Cells by Florescence Activated Cell Sorter Flow Cytometry. Cell cycle analysis of A549 lung cancer cell line treated with various concentrations (40 µg/ml and 80 µg/ml) of CCLE and CCRE for 24 hrs using Propidium Iodide

dye. Arrest of cells were observed with respect to untreated control A549 cells at S phase. Colchicine (25μM) was used as positive control. The data is represented in terms of percentage arrest. Data was statistically significant with $p < 0.05$ and was calculated using one way analysis of variance (ANOVA).

b. Percentage of A549 cells at various phases of cell cycle post treatment with *Chlorophytum comosum* leaves ethanolic (CCLE) and roots ethanolic (CCRE) extracts.

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