Anticancer potential of *Solanum lycopersicum* L. extract in human lung epithelial cancer cells A549

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The study aimed to reveal the phytochemical profile, free radical scavenging potential, and anticancer activity of *Solanum lycopersicum* L. leaf extract (SLLE). According to the study, SLLE contains plant secondary metabolites that are beneficial for health, like phenolics, flavonoids, ascorbic acid, alkaloids, and terpenoids. The SLLE has shown potential free radical scavenging potential in DPPH and ABTS free radical scavenging analysis and its EC\(_{50}\) values (concentration required to inhibit 50% of free radicals) were determined as 481.29 ± 33.82 and 527.56 ± 20.34 µg/mL, respectively. The SLLE has the ability to scavenge free radicals and could be used to treat illnesses brought on by oxidative stress. The anticancer activity of SLLE was assessed by MTT, LDH, micro-morphological, live/dead dual staining, and caspase-3 analysis. In the MTT assay, the IC\(_{50}\) value (concentration required to inhibit 50% of cell viability) was determined as 190.41 ± 4.77 µg/mL. Furthermore, SLLE has shown potential anticancer activity by adversely affecting the plasma membrane integrity and escalating the caspase-3 levels. In the biomedical field, SLLE could be highly useful to treat cancer.

**Keywords:** Anticancer activity, Antioxidant activity, Apoptosis, LDH assay, Live/dead dual staining, MTT assay, *Solanum lycopersicum* L.

Oxidative stress is intimately connected to every aspect of cancer, including tumorigenesis, therapy, and prevention. The oxidative stress that the human body is continually experiencing comes from both endogenous ([at the cellular level where mitochondria are involved] and external sources (UV light, pollutants, toxins, chemicals, etc). Intracellular signal transduction and transcription factors may be directly or indirectly impacted by oxidative stress when it surpasses the body's capacity for oxidation-reduction, which can result in gene alterations or carcinogenesis. It is also claimed that the tumor-bearing condition is under oxidative stress, which is linked to active oxygen synthesis by the tumor cells and aberrant oxidation-reduction control. Substantial research has demonstrated that ROS interacts with biological macromolecules like DNA, proteins, and lipids, impairing crucial physiological processes. Normal cells can turn into cancerous cells as a result of ROS-induced oxidative modifications such as DNA base changes, strand breakage, disruption to tumor suppressor genes, and activation of proto-oncogenes. A better comprehension of how ROS controls autophagy and apoptosis provides possibilities to establish ROS-inducing or -inhibiting cancer therapeutic techniques depending on the molecular setting and microenvironment of a particular malignancy. By researching the significance of enhanced ROS production in cancer, ROS-regulated signaling pathways, and identifying particular antioxidants as targets, it is now possible to develop specific and efficient medicines to target cancer cells\(^{1,2}\).

In this regard, researchers are actively studying the use of antioxidants and how well they can prevent the development of cancer. Compounds that slows or prevents the oxidation of substrate in low concentrations is referred to as an antioxidant. There are two types of antioxidants: endogenous and exogenous. The cofactors and enzymes that neutralize ROS are known as endogenous antioxidants. The three enzymatic systems, SOD, CAT, and glutathione peroxidase (GPx), are critical to the function of
biological systems’ endogenous antioxidant defenses against free radicals. A wide class of molecules known as exogenous antioxidants can be divided into three subclasses: polyphenols, vitamins and derivatives, and antioxidant minerals. Flavonoids and phenolic acids are subclasses of polyphenols, which are the most prevalent natural antioxidants. Catechins, flavones, flavanones, isoflavones, flavonols, and anthocyanins are further subtypes of flavonoids. Fruits, vegetables, juices, tea, wine, and other plant-based products contain polyphenols.\(^3\)\(^5\)

For many years, studies on the effects of plant bioactive components have mostly concentrated on how well they can prevent cancer. Today, it is clear why some foods have therapeutic effects and why eating fruits and vegetables may result in a decrease in the frequency of specific cancer entities. Numerous bioactive phytochemicals are present in plants, including flavonoids, polyphenolic compounds, caffeic acid, catechins, saponins, polysaccharides, triterpenoids, alkaloids, glycosides, phenols, eugenol, curcumin, carnosic acid, oleanolic acid, quercetin, luteolin, kaempferol and luteolin glycosides, rosmanic acid, emodin, etc. These bioactive substances can inhibit cell cycle checkpoints and promote apoptosis by activating initiator and executor caspase, two processes by which they can decrease tumor cell proliferation. Due to its anti-cancer effects, antioxidant qualities, anti-inflammatory characteristics, anti-mutagenic capabilities, and anti-angiogenic properties, traditional medicines have been utilized to treat cancers all over the world. Additionally, when given in their active and pure forms, these medications successfully reduce the early and intermediate stages of carcinogenesis.\(^7\)

In the present study, the ethanolic leaf extract of\(\textit{Solanum lycopersicum}\) L. was considered to evaluate the antioxidant and anticancer properties. The production of\(\textit{S. lycopersicum}\) fruit, which totals 170 million tonnes annually, is significant on a global scale. The potato is the most significant vegetable crop in the world, followed by tomatoes\(\textit{(S. lycopersicum)}\ L.). Over the past 25 years, tomato output and consumption have increased significantly worldwide. Approximately 170.75 million tonnes of fresh fruit are being produced worldwide on 5.02 million hectares of land in more than 150 different nations. The tomato plant has undergone selective breeding to increase yield and fruit quality. One of the most lucrative vegetable crops is the tomato due to its widespread use in processing and cooking.\(^8\)\(^9\).

In the present study,\(\textit{S. lycopersicum}\) L. leaf extract (SLLE) was obtained by cold maceration technique using ethanol as solvent. Following, qualitative and quantitative phytochemical profile was revealed. The antioxidant potential of SLLE was unveiled by DPPH and ABTS assays. The anticancer activity of SLLE was unveiled in A549 cells (human lung carcinoma epithelial cells) by various cell viability assays such as MTT, LDH, micromorphological observation, and live/dead staining assays.

Materials and Methods

Chemicals and reagents

Ferric chloride, distilled water, virgin olive oil, Molisch's reagent, ethyl acetate, ammonia, Molisch's reagent, concentrated sulfuric acid, Fehling solution, chloroform, Meyer's and Wagner's reagents, sodium bicarbonate, ferrous sulfate, iodine, and other chemicals used in the study were fine grade and they were obtained from Merck, Bengaluru, India. Dulbecco's modified Eagle medium, distilled water, dimethyl sulfoxide (DMSO), LDH assay kit, isopropanol (99%), Dulbecco's phosphate buffered saline (pH 7.4), live/dead dual staining assay kit, fetal bovine serum (FBS), 4′,6-diamidino-2-phenylindole, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl—tetrazolium bromide (MTT), and caspase-3 kit were obtained from Sigma-Aldrich, Bengaluru, India. The cell culture plasticware was obtained from Nunc, Bengaluru, India.

Plant material collection

\(\textit{S. lycopersicum}\) L. plants were collected from Guntur, identified, and a voucher specimen was safeguarded in the Department of Biotechnology, Acharya Nagarjuna University, Guntur, India. The leaves were detached from the plant and dried at room temperature under dark conditions. Following, 500 g of dried leaves were powdered using a blender and subjected to cold maceration using ethanol to obtain the extract.\(^10\) The obtained \(\textit{S. lycopersicum}\) L. leaves extract (SLLE) was subjected to filtration by Whatman no.1 filter paper and the filtrate was stored in the dark at -20°C, in vacuum bags, for further phytochemical analyses and other experiments.

Qualitative phytochemical profile

Test for Phenols

A test was conducted utilizing the approach of Sofowora.\(^11\) In a beaker, 2 mL of SLLE was taken. Then, 2 mL of a solution of ferric chloride was added. The presence of phenols was indicated by a deep bluish-green solution.
Test for Saponins
The approach developed by Edeoga et al. was used to conduct test\textsuperscript{12}. 2 mL of SLLE was boiled in a water bath with 20 mL of distilled water before filtering the mixture. In order to create a stable, long-lasting froth, 10 mL of the filtrate was combined with 5 mL of distilled water and forcefully shaken. Three drops of extra virgin olive oil were added to the foam, and the mixture was violently shaken to generate an emulsion that showed saponin presence.

Test for carbohydrates
The test was conducted as per the approach of Sofowora\textsuperscript{11}. Briefly, 2 mL of Molisch’s reagent and 3 mL of SLLE were mixed and agitated. Following, concentrated sulfuric acid (2 mL) was carefully added along the test tube’s side. A positive test result was indicated by the development of a red or dim violet tint at the interface between the two layers.

Test for flavonoids
Using the methodology of Harborne, the test was conducted\textsuperscript{13}. Ten mL of ethyl acetate was heated with 1 mL of SLLE for 5 min over a steam bath (40–50°C). Using 1 mL of diluted ammonia, the filtrate was treated. A golden color indicated that a test for flavonoids was successful.

Test for steroids
The technique reported by Edeoga et al. was used to identify steroids\textsuperscript{12}. 2 mL of concentrated sulfuric acid and 2 mL of acetic anhydride were added to 1 mL of SLLE, and the color changed from blue to dark green, indicating the presence of steroids.

Test for glycosides
The Parekh and Chanda approach was used to perform the Kellar-Kiliani test\textsuperscript{14}. 1 mL of Glacial acetic acid and 2 mL of SLLE were added. Then, 1 mL of strong sulfuric acid and 1 mL of ferric chloride were added. The presence of glycosides was shown by the solution’s green-blue color.

Test for terpenoids
The Salkowski test was carried out as per the approach of Edeoga et al\textsuperscript{12}. 2 mL of chloroform were combined with 5 mL of the SLLE. To generate a layer, 3 mL of concentrated sulfuric acid was then added. The presence of terpenoids was suggested by the interface’s reddish-brown color.

Test for free reducing sugars
Using the approach of Sofowora, an experiment was conducted\textsuperscript{15}. A crimson cuprous oxide precipitate formed after adding Fehling solution (0.5 mL) to SLLE (2 mL) and showed the presence of free-reducing sugars.

Test for tannins
A test was carried out utilizing the methodology of Sofowora\textsuperscript{11}. A volume of 2-3 mL of SLLE was mixed with 10% alcohol-based ferric chloride solution (1:1). The solution turned a dark blue tint, indicating that tannins were present.

Test for alkaloids
Using the Harborne, approach, the test was conducted\textsuperscript{13}. One mL of SLLE was added to 5 mL of 2N hydrochloric acid. Following that, Meyer’s and Wagner’s reagents were applied to the solution mixture. Turbidity was used to determine whether the samples were positive.

Test for Starch
Applying the methodology of Ganesan and Bhatt, a test was conducted\textsuperscript{15}. Using iodine as a reagent, the presence of starch in the sample was detected by a dark blue hue that developed when the SLLE sample was heated and then reappeared when it was cooled.

Test for ascorbic acid
Using Ganesan and Bhatt method, a test was conducted\textsuperscript{15}. 2 mL of SLLE, 2 mL of 2% w/v solution, 2 mL of water, 0.1 g of sodium bicarbonate, and around 20 mg of ferrous sulfate were added and shaken and left to stand for 30 min at room temperature. Following, when 5 mL of 1M sulfuric acid was added, the resulting deep violet color vanished and indicated the presence of ascorbic acid.

Quantitative phytochemical profile
Quantification of total flavonoid content
With slight adjustments, the Gunti et al. aluminium chloride colorimetric technique was used to determine the total flavonoid concentration in SLLE\textsuperscript{16}. A mixture of 1.5 mL of methanol and an aliquot of 0.5 to 2 mL of SLLE were combined. Then, 0.1 mL of 10% aluminum chloride, 0.1 mL of potassium acetate (1 M), and 2.8 mL of distilled water were added. For 30 min, the reaction mixture was left at room temperature. At 415 nm, the reaction mixture's absorbance was measured. Rutin served as a standard, and the calibration curve was produced using it. The results represented as mg of rutin equivalent/gram of SLLE and the total flavonoids were calculated.
Quantification of total phenolic content

Using the Folin-Ciocalteu reagent, the Kalagatur et al. technique was used to determine the total phenolic content. 0.1 mL to 1 mL of SLLE were combined with 0.75 mL of the Folin-Ciocalteu reagent in an aliquot (10-fold diluted with distilled water). After 5 min at room temperature, 0.75 mL of 6% sodium carbonate was added to the mixture. Its absorbance at 725 nm was measured after the procedure had taken 90 min. Gallic acid was used to draw the calibration curve for the standard. In terms of mg gallic acid equivalent/gram of SLLE, the total phenolics were expressed.

Quantification of free radical scavenging potential

DPPH assay

With some adjustments, the Kalagatur et al. method was used to test the SLLE capacity to scavenge free radicals. SLLE at various concentrations (0.50 to 5 mL) was combined with 950 mL of a DPPH methanolic solution (3.4 mg/100 mL). The reaction mixture was left at 37°C in the dark for one hr. The initial purple color’s elimination is a sign of the extracts’ capacity to scavenge free radicals. The reaction mixture’s absorbance was measured with a microplate reader at 517 nm (Synergy H1, BioTek, USA). The positive control used was ascorbic acid. The following formula was used to compute the DPPH scavenging capacity:

\[
\text{DPPH free radicals} \% = \left( \frac{C_{OD} - T_{OD}}{C_{OD}} \right) \times 100
\]

where, \(C_{OD}\) denotes the optical density of the control sample. \(T_{OD}\) denotes the optical density of the test sample.

ABTS assay

With slight adjustments, the method outlined by Kumar et al. was used to determine the ABTS radical scavenging activity of SLLE. The ABTS radical stock solution was made by mixing an equal amount of an aqueous ABTS solution (7 mM) with an aqueous potassium persulfate solution (2.45 mM) and letting them react for 12 to 16 h at room temperature in the dark. Then, 0.50 – 5 mL of the SLLE or standard (ascorbic acid) was combined with 1 mL of the ABTS radical solution. The mixture was then incubated for precisely 10 min in the dark at room temperature. The control was made by using 0.50 mL of double distilled water with 1 mL of ABTS radical solution. The control solution’s absorbance was measured to be between 0.38 and 0.04. The optical density was noticed at 734 nm using a microplate reader (Synergy H1, BioTek, USA). Using the equation below, the percentage results of ABTS scavenging activity were determined as % of inhibition.

\[
\text{ABTS free radicals} \% = \left( \frac{C_{OD} - T_{OD}}{C_{OD}} \right) \times 100
\]

where, \(C_{OD}\) denotes the optical density of the control sample. \(T_{OD}\) denotes the optical density of the test sample.

Anticancer activity

Cell culture and maintenance

The A549 cells (human lung carcinoma epithelial cells) were bought from the repository cell line center of the National Centre for Cell Science (NCCS), Pune, India. They have maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 µg/mL) at 37°C in a humidified environment with 5% CO₂.

MTT assay

The effect of SLLE on cell viability of cancer (A549) cells were revealed by MTT assay. In 96-well plates, cells (1 × 10⁵/well) were plated in 100 µL DMEM medium with 10% FBS in each well. Confluence was attained by the cells after 48 h of incubation. The cells were then treated for 48 h at 37°C with varying doses of the SLLE in 0.01% DMSO. Following, 20 µL/well (5 mg/mL) of 0.5% 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl—tetrazolium bromide (MTT) solution (prepared in Dulbecco’s phosphate buffered saline, pH 7.4) was added after the sample solution was removed, and washed with Dulbecco’s phosphate-buffered saline (pH 7.4). Next, 0.04 M of isopropanol was added after 4 h of incubation. The wells containing cells without samples as controls. The viability of the cells was identified by measuring formazan crystal absorbance at 570 nm using a microplate reader (Synergy H1, BioTek, USA). The concentration necessary to inhibit viability by 50% (IC₅₀) was computed graphically after measurements were made in triplicates. Using the following formula, the effect of the SLLE was quantified as the percentage of cell viability.

\[
\text{% of cell viability} = \left( \frac{C_{OD} - T_{OD}}{C_{OD}} \right) \times 100
\]
Where, \( C_{OD} \) denotes the optical density of the control sample, \( T_{OD} \) denotes the optical density of the test sample.

**LDH assay**
The effect of SLLE on cell viability of cancer (A549) cells were also revealed by another cell viability technique LDH assay\(^{22}\). Similar to the MTT assay, cells were seeded in a 96-well plate and treated with different concentrations of SLLE for 24 h. Following, LDH release was quantified using the kit, and instructions were followed as per the manufacturer of the kit.

**Micro-morphological observation**
The effect of SLLE on the micro-morphology of cancer (A549) cells were revealed by bright-field inverted microscopic observation\(^{23}\). In 6-well plate of cells was seeded with \( 1 \times 10^5 \) cells per well and left to develop for 2 days. The cells were exposed to different concentrations of SLLE for 24 h, morphological changes were evaluated to assess the alterations generated by SLLE. Following the incubation period, cells were washed in Dulbecco’s PBS (pH 7.4) and examined with a bright-field inverted microscope, and images were captured using an attached digital camera (Olympus IX51, Tokyo, Japan).

**Live/dead dual staining analysis**
The effect of SLLE on cell viability of cancer cells (A549) was revealed by live/dead dual staining analysis consisting of calcein AM and ethidium homodimer \(^1\). Similar to the MTT assay, cells were seeded in a 96-well plate and treated with different concentrations of SLLE for 24 h. Cells treated with DMEM medium devoid of FBS and SLLE served as the control. After a 24 h incubation period, cells were gently washed twice with D-PBS before being stained using a live/dead dual staining kit in accordance with the manufacturer's instructions. By using the suggested manufacturing formula, the living and dead cells contained in the experiment were calculated. Additionally, using an EVOS FLC fluorescent microscope, fluorescent microscopic images were taken using red fluorescent and green fluorescent protein filters (Thermo Fisher Scientific, USA).

**Apoptosis analysis**
The effect of SLLE on apoptosis was revealed by estimating caspase-3 levels\(^{25}\). The cells were plated and treated with different concentrations of SLLE as mentioned in the MTT analysis. Following the 24 h treatment with the aforementioned techniques, the cells were washed twice with Dulbecco’s PBS. The cells were collected by trypsinization and centrifugation at 6000 rpm for 4 min at 4°C. The Bradford assay was used to quantify the protein quantity. Then, utilizing DEVD-pNA as a specific substrate for caspase-3, samples containing 250 g of total protein were checked for their presence. The absorbance was measured at 405 nm using a plate reader (Synergy H1, BioTek, USA). Results from the study were reported with respect to the control (a fold of control).

**Statistical analysis**
Triplicates of each experiment were performed. Mean ± SD was used to represent experimental data. By using Analysis of Variance, the data were examined for statistical significance. At \( P \leq 0.05 \), data were considered significant.

**Results and Discussion**

**Phytochemical analysis**
Through phytochemical screening, different plant compounds are isolated in order to evaluate their biological activity or potential as medicines. The presence of certain chemicals in plants that have a clear physiological impact on the living system is what gives them their therapeutic values. In the present study, the qualitative phytochemical profile of SLLE was evaluated which includes carbohydrates, reducing sugars, starch, phenols, flavonoids, glucosides, terpenoids, alkaloids, tannins, and ascorbic acid (Table 1). The results showed that SLLE was quite rich in useful molecules such as phenols, flavonoids, glucosides, terpenoids, and treated with different concentrations of SLLE as mentioned in the MTT analysis. Following the 24 h treatment with the aforementioned techniques, the cells were washed twice with Dulbecco’s PBS. The cells were collected by trypsinization and centrifugation at 6000 rpm for 4 min at 4°C. The Bradford assay was used to quantify the protein quantity. Then, utilizing DEVD-pNA as a specific substrate for caspase-3, samples containing 250 g of total protein were checked for their presence. The absorbance was measured at 405 nm using a plate reader (Synergy H1, BioTek, USA). Results from the study were reported with respect to the control (a fold of control).

The results showed that SLLE contains potential phenolics and flavonoids, and they were found to be dose-dependent with the dose of SLLE (Fig. 1). The quantity of total phenolics and flavonoids was determined as 12.04 ± 0.16 mg/g gallic acid equivalents and 15.89 ± 0.23 mg/g of rutin equivalents, respectively. The study revealed that SLLE contains potential health-beneficial plant secondary metabolites. Especially, SLLEE was found to contain a good amount of beneficial phenolics and flavonoids.
In support of our study, Kainat et al. have S. lycopersicum L. peel and pulp tested for phytochemicals, and it was found that both have a large number of phytochemicals. The ethanolic and aqueous extracts of the peels and pulp are both high in phenolics, flavonoids, tannins, alkaloids, and terpenoids. The majority of a plant's secondary metabolites are phenolics, which are widely diversified throughout the plant kingdom. Due to their strong antioxidant qualities and notable impacts in the prevention of numerous oxidative stress-related disorders, such as cancer, plant polyphenols have gained growing attention. Furthermore, today, flavonoids are regarded as a crucial ingredient in a wide range of medicinal, nutraceutical, pharmaceutical, and cosmetic applications. This is explained by their ability to influence the activity of essential cellular enzymes as well as their anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic capabilities.

Free radical scavenging potential analysis

Free radicals are unstable molecules that the body produces as a response to environmental and other stressors. Antioxidants are compounds that can stop or slow cell damage caused by these unstable molecules. Antioxidants can derive from either natural or synthetic sources. Antioxidants are known to be abundant in several plant-based foods. Antioxidants derived from plants are a type of phytonutrient or nutrient derived from plants.

In the present study, the free radical scavenging potential of SLLE was determined by DPPH and ABTS assays. The tested SLLE showed dose-dependent free radical scavenging potential in DPPH and ABTS free radical scavenging assays (Fig. 2). The EC50 value (concentration required to inhibit 50% of free radicals) of SLLE in DPPH and ABTS assay was determined as 481.29 ± 33.82 and 527.56 ± 20.34 µg/mL, respectively. In support of the study, Bhandari & Lee have determined the antioxidant profile and proved that the antioxidant potential of S. lycopersicum L. is due to relatively higher levels of carotenoids, ascorbic acid, total phenols, and flavonoids. In our study too, we have determined phenolics, flavonoids, ascorbic acid, and other secondary metabolites which responsible to antioxidant activity. The study revealed that SLLE could be useful to ameliorate the oxidative stress mediated diseases like cancer.

Anticancer activity

Anticancer activity of SLLE on A549 cells was revealed by different assays such as MTT, LDH, micro-morphological observation, live/dead dual staining, and caspase-3 analysis.

The MTT assay is based on the measurement of mitochondrial activity by formazan crystal formation from MTT by live cells. This assay is often used to assess the in vitro cytotoxic effects of medicines/cancer drugs on cell lines since for the majority of cell populations the total mitochondrial activity is correlated with the proportion of...
In the present study, SLLE has dose-dependently inhibited the viability of the A549 cancer cells (Fig. 3). The IC$_{50}$ value (concentration required to inhibit 50% of cell viability) of SLLE and cisplatin was determined as 190.41 ± 4.77 µg/mL and 5.20 ± 0.09 µM, respectively. The assay concluded that SLLE has limited the cell viability of A549 cancer cells.

The anticancer activity of SLLE was determined as a measurement of lactate dehydrogenase (LDH). All cells contain the stable cytoplasmic enzyme LDH. The LDH is quickly released into the cell culture supernatant when the plasma membrane is disrupted, a crucial aspect of cells going through apoptosis, necrosis, and other types of cellular destruction. In the present study, SLLE has dose-dependently escalated the level of LDH in culture medium (Fig. 4). The study concluded that SLLE has induced the cell death of cancer cells through damaging the plasma membrane integrity.

The anticancer activity of SLLE was further confirmed by observing the micro-morphology of A549 cells (Fig. 5). The healthy morphology of the control cells was observed to have a smooth surface and distinctive form. Contrarily, cells treated with IC$_{50}$ levels of SLLE (190.41 ± 4.77 µg/mL) and cisplatin (5.20 ± 0.09 µM) revealed adverse alterations in the micro-morphology, including the loss of the monolayer, the advent of cell debris, the development of apoptotic bodies, and a reduction in cell number. The study demonstrated that SLLE adversely induced micro-morphological alterations, which had an impact on the viability of the cells. The observed outcomes were discovered to be consistent.
The anticancer effect of SLLE was further assessed by other cell viability assay live/dead dual staining technique. The stain kit contains ethidium homodimer-I and calcein AM. A green dye called calcein AM indicates the presence of living cells. Dead cells are indicated by the red color of the ethidium homodimer-I stain. The live/dead dual staining depends on the integrity of the membrane; cells with compromised or broken membranes appear red and are classified as dead cells. The live/dead cell assay concluded that SLLE induced the cell death through damaging the cell membrane integrity of A549 cancer cells.

Important mediators of programmed cell death (apoptosis) are caspases. Among these, caspase-3 is a death protease that is regularly activated and catalyzes the precise cleavage of numerous essential cellular proteins. Caspase-3 is necessary for all cell types under investigation for apoptotic chromatin condensation and DNA fragmentation, as well as certain other common apoptotic markers. As a result, caspase-3 is crucial for some processes involved in cell death and the production of apoptotic bodies. In the present study, SLLE has dose-dependently escalated the caspase-3 levels. The study showed that SLLE has induced the cell death through apoptosis and obtained results were in line with the outcome of MTT, LDH, micro-morphological, and live/dead dual staining analysis.

In support of our study, researchers have proved that plant extracts have potential anticancer activity. Prior research has shown that phenols, flavonoids, terpenoids, and alkaloids have the capacity to display antioxidant, anti-inflammatory, and anticancer activity which causes them to have positive health impacts. The primary mechanisms by which plant secondary compounds exert their anti-carcinogenic effects include their capacity to: cause cell cycle arrest; boost tumor suppressor proteins like p53; block oncogenic signaling cascades that regulate cell proliferation, angiogenesis, and apoptosis; adjust ROS levels; and enhance the capacity to differentiate and transform into normal cells. In our study, SLLE has shown potential anticancer due to the attendance of phenols, flavonoids, terpenoids, and alkaloids.
Conclusion

The study concluded that SLLE contains health-beneficial plant secondary metabolites such as phenolics, flavonoids, ascorbic acid, alkaloids, and terpenoids. The SLLE has potential free radical scavenging potential and could be used to overcome oxidative stress-related diseases. The SLLE has shown potential anticancer activity on A549 cancer cells by detrimentally affecting the cell membrane, inhibiting metabolic activity, and escalating the caspase-3 levels. The SLLE could be potentially applicable as an anticancer agent in the biomedical field.

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Conflict of interest

All authors declare no conflict of interest.

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