

## COMPARATIVE PHYTOCHEMICAL CHARACTERIZATION AND ANTICANCER ACTIVITY OF *MENTHA SPICATA*, *ZIZIPHUS SPINA-CHRISTI*, *THYMUS VULGARIS*, *SALVIA ROSMARINUS*, *OCIMUM BASILICUM*, AND *AZADIRACHTA INDICA* LEAVES AGAINST A549 LUNG CANCER CELLS

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### Abstract

Chronic exposure to environmental carcinogens, mostly tobacco smoke, causes progressive genetic mutations and epigenetic changes in the bronchial epithelium, contributing to the multifactorial etiology of lung cancer. To treat this cancer subtype, new anti-cancer drugs still need to be discovered. The ethanolic extracts (70%) of fresh plant leaves consisting of mint, sidr, thyme, rosemary, basil, and neem were used compared with cisplatin as a positive control to treat A549 lung cancer cells. Cell viability, redox state, inflammation, hormonal signaling, and apoptotic pathway tests were used to assess biological activities. The extracts of neem and thyme showed more cytotoxicity at 200 µg/mL than cisplatin (10 µg/mL), lowering cell viability to 20.29% and 25.37%, respectively, whereas cisplatin reached 40.33%. Cisplatin and neem dramatically increased indicators including reactive oxygen species (ROS) (263.25%), nitric oxide (NO) (23.57 µM), and malondialdehyde (MDA) (8.14 nmol/mg) in conjunction with this anti-proliferative action, which was linked to an elevation of oxidative stress. Additionally, both therapies significantly reduced the cellular antioxidant defense, with thiol content falling to 40.36 % (neem) and 36.25% (cisplatin). At the same time, they inhibited important enzymatic activities, such as glutathione peroxidase (GPx) (44.39% vs. 35.2%), superoxide dismutase (SOD) (45.36% vs. 41.36%), and catalase (CAT) (43.04% vs. 36.12%). Neem extract and cisplatin treatments significantly increased tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (33.67 and 39.44 pg/mL) and interleukin-6 (IL-6) (38.51 and 34.21 pg/mL), which in turn caused apoptosis. Neem caused early (25.87%) and late (21.01%) apoptosis, which is similar to the 27.05% and 27.14% that cisplatin caused. Reduced BCL-2 and BCL-XL to as low as 37.22% and 41.22%, respectively, and increased caspase-9 and caspase-3 activity from a control baseline of approximately 0.85 to peaks of 3.25 and 3.89 U/mg protein. Additionally, both medicines demonstrated the strongest reductions in dihydrotestosterone (DHT) and prostate-specific antigen (PSA) levels, demonstrating their strong hormonal inhibitory and anti-proliferative properties. In contrast to basil and rosemary, which were less efficient, mint and sidr demonstrated considerable cytotoxicity (viability 45–55%). By lowering thiols to almost 55%, rosemary and thyme upset the redox equilibrium, whereas basil was the only one to lower CAT activity to 36.06%. All extracts reduced PSA/DHT levels and somewhat raised early apoptosis (10–15%), but their total bioactivity was still much less than that of the neem and cisplatin treatments. The current findings lend credence to leafy green plant's especially neem and thyme possible use in treating A549 lung cancer and serve as the foundation for further research.

**Key words:** Natural Anticancer Leaves; Cytotoxicity; A549 Lung Cancer Cell Line; Apoptosis Activity; Cisplatin

## Introduction

Worldwide, the lung cancer disease is the one of the principal reasons of cancer-related mortality (Abushal *et al.*, 2026). Radiation treatment, chemotherapy, surgery, or a combination of these are common forms of treatment. Patients frequently have harmful side effects from these costly, severe therapy alternatives (Al-Eisa *et al.*, 2023). Lung cancer disease is well-known as the irregular and uncontrolled proliferations of the malignant cells within the pulmonic manner. The process basically activated by a combined effect of continuing tobacco smoking in charge for over 80% of cases, ecological exposure to hazards such as radon and asbestos, and genomic mutations in pathways (Sambo *et al.*, 2025). Therefore, the needs to implement low-toxicity, active cancer treatments, which are frequently found in natural products. Natural products are regarded as great resource for the development of new anticancer medicines because of their many therapeutic advantages. Utilizing natural products as spices, fruits, and vegetables to kill cacogenic cells and enhance general health has become a growing area of research in the field of cancer (Ahmed *et al.*, 2022b). There has been a recent boom in the search for complementary and alternative medications derived from natural sources. Epidemiological research has repeatedly indicated a negative correlation between consumption of fruit and vegetable and the risks of cancer (Ishfaq *et al.*, 2022). The synergistic and additive mixture of secondary metabolites that are physiologically active as alkaloid, flavonoid, terpenoid, essential oil, and glycoside, that are contemporary in fruits and vegetables and have anticarcinogenic qualities has been related to these health benefits (Zahara *et al.*, 2022). These characteristics are mediated by a number of pathways, including immunological activation, DNA repair system stimulation, and altered carcinogen metabolism (Li *et al.*, 2014). The benefits of employing these plant-derived substances include their availability in a consumable form and comparatively low toxicity (Rokayya *et al.*, 2013). Peppermint or mint (*Mentha spicata*) from Lamiaceae family, has been used safely for a long time as a flavoring and in medical formulations. Several investigations have shown that mint leaf oil and aqueous extract have chemopreventive and radioprotective properties (Ashraf *et al.*, 2025). However, the majority of these benefits were ascribed to protective action against radiation-induced changes in serum phosphatase activities, lipid peroxidation, and hematological components of peripheral blood (Deepika *et al.*, 2011). Antioxidant capacity, apoptosis induction, cancer cell cytotoxicity, target selectivity, and inhibition of cancer cell growth are some of the anti-cancer qualities for mint leaves (Aldoghachi *et al.*, 2022).

Sidr (*Ziziphus spina-christi*), is a member of the Rhamnaceae family (Kadi *et al.*, 2025). Mongalo *et al.*, (2023) reported that the leaves contained large levels of total phenolics content, flavonoids, with high contents of quercetin, terpenoid, quinone, and alkaloid. Furthermore, the roots and leaves have been utilized to treat a number of illnesses, such as rheumatic pains, fever, ear inflammation, syphilis, gonorrhea, measles, cancer, ear inflammation, tumors, cough, chest complaint, dysentery, sore, glandular swelling, skin disease, open and swollen wounds (Almehmadi *et al.*, 2025). One promising strategy for improvements in cancer treatment is the investigation of plant chemicals with particular qualities to inhibit protein kinases (Shahwar *et al.*, 2024).

Thyme (*Thymus vulgaris*) from Lamiaceae family is a herb that is frequently used in cooking and medicine because it contains bioactive chemicals that have antioxidant properties. Numerous illnesses have been demonstrated to be treated by thyme extracts, essential oil, and their refined or synthetic ingredients (Kamalia & Tunjung, 2023). Thymol can cause apoptosis and stop the growth of colorectal cancer cells (Zeng *et al.*, 2020). Significant cytotoxicity and cytogenic effects were demonstrated by thyme extracts, which also caused cell cycle arrest in some malignancies (Adham *et al.*, 2020). Additionally, thyme methanol extract has been shown to shield DNA from the damaging effects of chemotherapy.

Rosemary (*Salvia Rosmarinus*) is a member of the Lamiaceae family (Bahri *et al.*, 2017). According to Mengoni *et al.*, (2011), rosemary also contains additional derivatives with anti-inflammatory and anti-tumor properties, including ursolic acid, oleanolic acid, and micromeric acid.

Basil (*Ocimum basilicum*) from Labiatae family, is thought to be active due to a number of physiologically active components, as cardiac glycoside, terpenoid, alkaloid, flavonoid, saponin, steroid, tannin, and anthraquinones (Huang *et al.*, 2020). The development of prostate cancer cells was suppressed by aqueous and fractionated leaf extracts. Over the past ten years, studies on liver, breast, osteosarcoma, and cervical cell lines have also shown the anti-cancer effects of basil extracts (Curtis *et al.*, 2024).

Neem (*Azadirachta indica*) is a member of Meliaceae family. Numerous pharmacological actions, such as anti-inflammatory, antioxidant, antibacterial and antiviral, have been documented for neem leaves. Nimbin, nimbidine, isomeldenin,  $\beta$ -sitosterol, and quercetin are among the active ingredients found in neem leaves (Madhavan, 2021). The anticancer effects of the neem extract could be attributed to the reported phytochemicals of the extract, but compound characterization at the individual level is required to confirm the contribution of individual compounds like nimbidin. It has been demonstrated that quercetin,  $\beta$ -sitosterol, and nimbidine, have anti-inflammatory properties. Neem leaf extract has recently been shown to shield mice exposed to lipopolysaccharide (LPS) from endotoxemia (Lee *et al.*, 2017).

For creating intra- and inter-strand DNA cross-links, cisplatin effectively inhibits transcription and DNA replication at this specific dose. Apoptosis, or programmed cell death, is caused by signal transduction pathways that are triggered by this molecular interference. These pathways mainly involve p53 activation and mitochondrial cytochrome c release (Liao *et al.*, 2020). Figure 1. presents the chemical formula and 3D molecular structure of cisplatin.

Through the use of cisplatin, we were able to provide a clear baseline of high-efficacy growth inhibition, which made it possible to calibrate the evaluation of the plant extracts' anti-proliferative potency in comparison to a clinically approved chemotherapeutic treatment in A549 lung cancer cell line. Lung cancer is still a significant health problem worldwide, and traditional treatment methods are known to have low success rates and side effects. Today, a large number of medicinal plants have shown anti-cancer property, but there are not as many systematic comparative studies exploring the effect of multiple extracts on lung cancer cells, which also take into account cell toxicity, oxidative stress, inflammatory and apoptotic mechanisms. Thus, there is a need for more research to be conducted in order to find potentially useful plant derived candidates and to understand the mechanism of these against lung cancer.

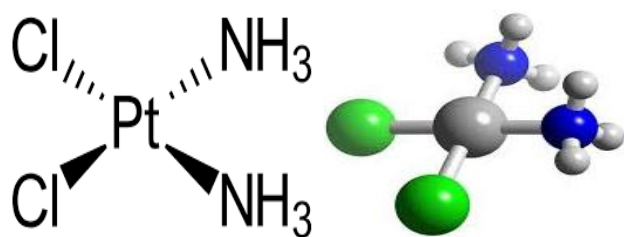


Fig. 1. Chemical formula and 3D molecular structure of cisplatin.

## Material and Methods

**Culturing of cell line:** The Shanghai Institute of Biological Sciences in China provided the A549 (human adenocarcinoma) cell lines. At 37°C and a humidified atmosphere with 5% CO<sub>2</sub> atmosphere, cells were seeded into 96-well at a density of ( $1 \times 10^4$  cells mL<sup>-1</sup>) and cultivated in Dulbecco's modified eagle medium (DMEM) media supplemented with 10% foetal bovine serum (FBS), and 1% antibiotic solution (100 U/mL penicillin and 100 U/mL streptomycin). The cells were cultivated until they were 80–90% confluent, the cell monolayer was washed with phosphate-buffered saline (PBS) and DMEM was aspirated and replenished every 48 hours. The cells were incubated with 0.25% trypsin-EDTA in order to perform cell detachment (Shahwar *et al.*, 2024)

**Plant materials and treatments:** The fresh plant leaves consisting of mint, sidr, thyme, rosemary, basil, and neem were harvested from some Taif City farms, Saudi Arabia by cutting off stems. A practitioner of indigenous knowledge personally selected the plant, while leaves were picked from stems and taxonomically identified by the Biotechnology Department at Taif University. After washing with distilled water to completely clean the leaves and get rid of dust and other foreign objects were turned every day and allowed to dry up in the shade at room temperature to maximize drying capacity and stop the growth of mold. The leaves were ground into powder by a conventional blender. It was subsequently sieved to get rid of any undesirable woody fibers until they were ready to use, the powdered leaves were kept at 4°C (Curtis *et al.*, 2024).

**Preparation of plant extract stock solutions:** A Whatmann number 1 filter paper was used to filter the homogenate after a 50 g sample of dried plant powder was soaked in 250 mL of 70% ethanol with 1: 5 (w/v) plant: solvent ratio for 72 hours by shaking at 150 rpm on a rocking platform in the dark condition to create a homogenous extract. The components in the plant-based extract were not harmed by soaking. A rotary evaporator was used at 40°C for filtering and concentration under low pressure after extraction using the maceration method. After being lyophilized, the resultant crude extracts were freeze dried and kept at -20°C until they could be examined further (Eftekhari *et al.*, 2022). To create a concentrated stock solution (80 mg/mL), the dried plant extracts were dissolved in Dimethyl sulfoxide (DMSO), vortexed until fully dissolved, and then sterilized using a 0.22 µm syringe filter. Until it was used, the stock was kept at -20°C protected from light until further use. The stock solution was diluted in DMEM with 10% FBS to create working concentrations for cancer cell assays. The final DMSO concentration in the wells was kept below 0.1% to prevent solvent-induced cytotoxicity (Molina-Romo *et al.*, 2018).

**Experimental treatment design:** Briefly, in a 96-well tissue culture plate, A549 cells were planted at a density of  $1 \times 10^3$  cells/well and incubated 72 hours, the cells were exposed to plant extracts at escalating concentrations (25, 50, 100, and 200 µg/mL) to estimate the cytotoxic and anti-proliferative properties compared with 10 µg/mL cisplatin. To assess the extracts' highest anti-proliferative ability against the A549 cells, a dosage of 200 µg/mL was used as the upper experimental limit. Untreated cells were used as the negative control, while 10 µg/mL cisplatin (PHR1624 from Sigma Aldrich, USA) was used as the positive control to establish a standard baseline for comparison. Since the concentration ranges tested for cisplatin (10 µg/mL) and plant extracts (25–200 µg/mL) are different, it is not appropriate to compare the two for potency; quantitative comparisons should be made using IC<sub>50</sub> values determined under identical experimental conditions. Figure 2 presents the summary of green leafy extracts versus the cisplatin by investigating the viability, oxidative stress, and apoptosis on A549 cells.

**Cell proliferation assay:** The MTT assay, which is based on the reduction of the yellow tetrazolium salt MTT to purple formazan crystals by metabolically active cells, was used to measure cell viability after treatment. In summary, each well received 20 µL of MTT solution (5 mg/mL in PBS; Sigma-Aldrich, USA) and was incubated at 37°C for 4 hours. Following incubation, the MTT solution-containing culture media was withdrawn, and each well received 200 µL of DMSO to dissolve the formazan crystals that had formed. A microplate reader (RT 2100C, Shenzhen, China) was used to measure the absorbance at 570 nm. In comparison to the vehicle-treated control, which was regarded as 100%, cell viability was expressed as a percentage (Kamiloglu *et al.*, 2020).

**Reactive oxygen species (ROS):** In order to evaluate early cellular responses and biochemical changes without producing severe cytotoxicity, the tests employed a concentration of 25 µg/mL. This allowed for a clearer assessment of the extracts' effects on cell health and apoptotic pathways (Kamiloglu *et al.*, 2020). After the A549 cells were quantified with the non-fluorescent day 20 µM 2',7'-dichlorofluorescein diacetate (DCFH-DA) kit (D6883, Sigma Aldrich, USA) for 10 minutes at 37°C in the dark, ROS levels were measured using a microplate reader to quantify fluorescence intensity (Lee *et al.*, 2017). After removing any excess dye, the cells were again washed with PBS. A microplate reader was used to measure the fluorescence intensity at a 490/520 nm filter (Lee *et al.*, 2025). Normalized fluorescence (as % of control) was used to express ROS data.

**Nitric oxide (NO) production:** The stable end-product was measured to determine the amount of nitric oxide produced. Nitrite was assessed using the Griess reaction. After a 48-hour of treatment, 100 µL Griess reagent (Sigma-Aldrich, USA) (0.5% sulfanilamide, 2.5% H<sub>3</sub>PO<sub>4</sub>, and 0.05% naphthyl ethylene diamine in H<sub>2</sub>O) was mixed with 100 µL of medium in a 96-well plate and left to remain at room temperature for 10 minutes in the dark. Measurements of absorbance against sodium nitrite at a concentration of (0–100 µM) were made at 540 nm, and the results were reported in µM of nitrite (Bontempo *et al.*, 2021).

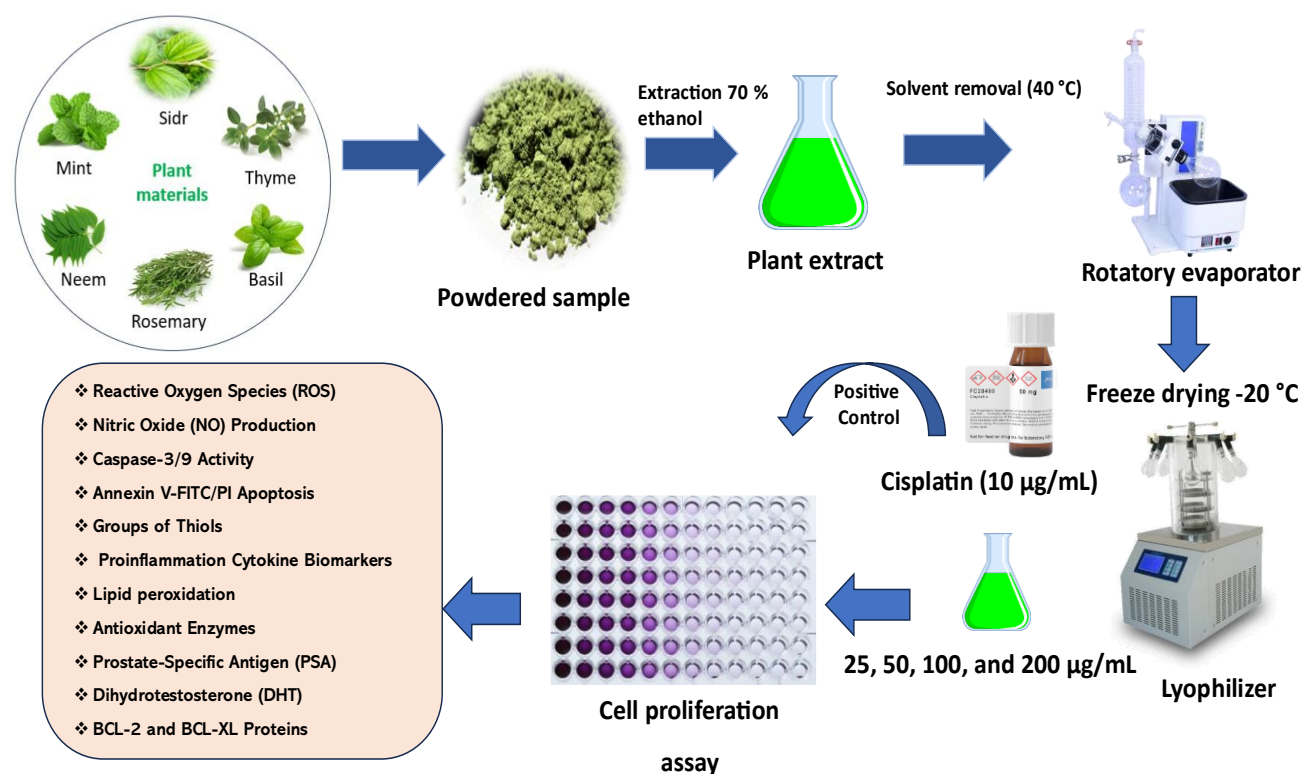


Fig. 2. The summary of the experimental design.

**Caspase-3/9 activity:** Using colorimetric assay kits (CASP3C and CASP9C from Sigma-Aldrich, USA), the activities of caspase-3 and -9 were measured in accordance with the manufacturer's instructions, respectively. Plant extracts (25 µg/mL) and cisplatin were applied to A549 cells grown in 96-well plates ( $1 \times 10^4$  cells/well) for 24-hour. Cells were lysed after incubation, and the supernatants were gathered. The lysates were treated for 2-hours at 37°C with the colorimetric substrates Ac-DEVD-pNA (for caspase-3) and Ac-LEHD-pNA (for caspase-9). A microplate reader was used to measure the release of the yellow chromophore p-nitroaniline (pNA) with an absorbance of 405 nm. The bicinchoninic acid protein assay (BCA) was used to determine the total protein content in order to standardize the enzymatic activity. The results were then reported as units per milligram of protein (U/mg protein) (Smith *et al.*, 1985).

**Annexin V-FITC/PI apoptosis:** Following treatment with plant extracts and cisplatin in compliance with the manufacturer's instructions, the rate of apoptosis in A549 cells was assessed using an Annexin V-FITC/PI apoptosis detection kit (APOAF, Sigma-Aldrich, USA). A549 cells in 6-well plates were exposed to each sample ( $1 \times 10^6$  cells/well) for 24-hours. Both adherent and floating cells were collected after incubation, washed three times with cold PBS, and then resuspended in 100 µL of 1× binding buffer. After that, 5 µL of Annexin V-FITC and 5 µL of propidium iodide (PI) were added to each sample. For fifteen minutes, the solutions were left to stand at room temperature in the dark. Fluorescence was measured using a microplate reader (HEALES MB-580, Shenzhen, China) equipped with specific optical filters suitable for FITC and PI detection. The instrument was set up to detect early apoptotic cells for FITC (Annexin V) at an excitation

wavelength of 488 nm and an emission wavelength of 530 nm (Muthu *et al.*, 2025). The excitation wavelength of PI, which stands for late apoptotic or necrotic cells, was 535 nm, whereas the emission wavelength was 617 nm. Black 96-well plates were used for all tests at room temperature to reduce background fluorescence. The recorded fluorescence intensities for FITC and PI were used to quantify the populations of early and late apoptotic cells in comparison to untreated control samples. The population's early and late apoptotic cell percentages were calculated and expressed in percentage.

**Groups of thiols:** Thiol groups have a role in the organs' detoxifying processes. This assay uses the DTNB (5,5-dithiobis-2-nitrobenzoic acid) in a redox reaction. The protein's free thiol (SH) converts DTNB disulphide to the yellow-u thiolate anion TNB [5-thio acid (2-nitrobenzoic acid)] at basic pH. Cell lysate in an amount of 0.05 mL was added to 1 mL of phosphate buffer, the absorbance (k1) at 412 nm was measured right away. After that, a second measurement of the absorbance (k2) was made by adding 0.02 mL of DTNB and letting it sit at room temperature for 15 minutes. Under the same circumstances, a background tube filled solely with DTNB solution is measured (Bahri *et al.*, 2017).

**Proinflammation cytokine biomarkers:** A549 cells were adjusted to a density of  $5.0 \times 10^2$  cells/well and then suspended in DMEM supplemented with 10% FBS. The cells were kept in a humidified atmosphere with 5% CO<sub>2</sub> at 37°C for 48-hours. Commercial ELISA kits, including those for tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (RAB0308 and RAB0477 Sigma-Aldrich, USA), were used to quantify the cytokines in the culture supernatants after incubation. Absorbance was

measured at 450 nm using an ELISA microplate reader (BK EL10A, Shandong, China), and the data were reported as pg/mL (Lee *et al.*, 2017).

**Lipid peroxidation:** The identification of thiobarbituric acid (TBA) reactive products, was used to measure lipid peroxidation. The oxidative degradation of lipids and polyunsaturated fatty acids produces free malondialdehyde (MDA), which can be detected in trace amounts by reaction with TBA. A549 cells were cultivated in a monolayer on 24-well plates. Cell lysate in an amount of 0.05 mL was added to and sodium phosphate buffer were both incubated for one hour at 37°C. The mixture was then precipitated with 10 % trichloroacetic acid (TCA) and centrifuged at 2,500 rpm. After that, we added 1% TBA and boiled the mixture for 15 minutes. With a molar extinction value of 156,000 M<sup>-1</sup>cm<sup>-1</sup>, colorimetric analysis was used at 532 nm and converted to nmol/mg protein (Bahri *et al.*, 2017).

**Antioxidant enzymes:** In accordance with the manufacturer's instructions, superoxide dismutase (SOD), which transforms superoxide radicals into oxygen and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The breakdown of H<sub>2</sub>O<sub>2</sub> into molecular oxygen and water is catalyzed by catalase (CAT). The oxidation of NADPH to NADP<sup>+</sup> in a coupled process, where GPx uses GSH to decrease peroxides and glutathione reductase uses NADPH to produce GSH from GSSG, was used to calculate GPx activity. They were chemically detected in triplicate using commercial assay kits (MAK196, MAK080, and CGP1 from Sigma-Aldrich, USA) at 450 nm, 520 nm, and 340 nm, respectively. Percentage was used to express the spectrophotometric method's results (Almeer *et al.*, 2019).

**Prostate-specific antigen (PSA) and dihydrotestosterone (DHT):** According to the manufacturer's instructions, commercially available ELISA kits (KA0210 and D-073) were used to measure the amounts of PSA and DHT in A549 cells, respectively. Cells were seeded at a density 1 × 10<sup>4</sup> cells/well and stimulated with 10 nmol/L DHT for 24 hours, with plant extracts and cisplatin. After incubation, the culture medium were gathered and centrifuged for 10 minutes at 2,000 rpm to get rid of any debris. A competitive ELISA method was used to assess DHT, while a sandwich ELISA methodology was used for the PSA assay. Tetramethylbenzidine TMB (3, 3', 5, 5'-Tetramethylbenzidine) substrate was added to start the enzymatic reaction. A stop solution was then added, and the intensity of the resulting yellow hue was quantified at 450 nm using a microplate reader. The results were reported as ng/mL for PSA and pg/mL for DHT (Wu *et al.*, 2014).

**BCL-2 and BCL-XL protein quantification:** Human-specific colorimetric ELISA kits (EKC32841-96T and EKA50059-96T) were used to assess the protein expression levels of BCL-2 and BCL-XL in accordance with the manufacturer's instructions. Plant extracts and cisplatin were applied to A549 cells for 24 hours, followed by lysis and centrifugation (10,000 rpm, 15 min, 4°C). 96-well plates coated with antibodies were supplemented with equal amounts of protein. TMB substrate was added after

the reaction was stopped with H<sub>2</sub>SO<sub>4</sub> and incubated with biotinylated detection antibodies and streptavidin-HRP reagent. The absorbance was measured at 450 nm, and the results were reported as a percentage of the untreated cells (Saeed *et al.*, 2025).

### Statistical analysis

For statistical analysis, IBM<sub>R</sub> SPSS<sub>R</sub> software version 29.0.0.0 was utilized. Tukey's post-hoc test was used to detect the significance after a one-way analysis of variance (ANOVA) test was used to examine the differences between the groups. When the p-value was less than 0.05, statistical significance was taken into consideration. The mean ± standard error of the mean (SEM) was used to display the data. Three independent replicates (n = 3) were used for each experiment.

### Results and Discussion

**The Effect of leafy extracts versus the cisplatin on cell viability of lung cancer cells:** The comparison data demonstrate these natural extracts' potential as complementary and alternative agents to traditional chemotherapy against lung cancer. According to the findings, every green leafy extract that was tested decreased the viability of A549 lung cells in a way that was dose-dependent, Fig. 3. At 200 µg/mL, neem and thyme extracts showed the most cytotoxic effects, lowering cell viability to 20.29% and 25.37%, respectively. At the same concentration, these extracts were more effective than those of mint, sidr, rosemary, and basil. Cell viability was decreased to 40.33% by cisplatin at 10 µg/mL, suggesting that some plant extracts, especially neem and thyme, had cytotoxicity that was on par with or higher than cisplatin at the tested levels. The results of the present study revealed that the extracts were not more potent than cisplatin as they were tested at a lower concentration, but neem and thyme had higher A549 cell viability reduction at the tested concentrations. Although additional investigations are needed to evaluate the precise mechanisms, these results imply that some green leafy extracts may cause apoptosis and limit lung cancer cell proliferation just as well as or even more effectively as cisplatin (Noorbazargan *et al.*, 2021). It is crucial to remember that cisplatin's well-established apoptotic mechanisms and therapeutic effectiveness continue to serve as standards for assessing these natural substances (Mokhtari *et al.*, 2023). Neem and thyme showed the most noticeable decrease in cell viability, outperforming cisplatin at the tested dosages, among the green leafy extracts that showed various degrees of cytotoxicity against A549 lung cancer cells. This implies that these natural extracts might work in ways different from or in addition to cisplatin to cause apoptosis and stop the growth of cancer cells. By modifying apoptotic mechanisms, including as caspase activation and cell cycle arrest, and lowering chemotherapeutic adverse effects, plant-derived drugs have been shown in multiple studies to improve cisplatin efficacy or overcome resistance (Di *et al.*, 2025). These results encourage more research into green leafy extracts as supplements or substitutes for traditional chemotherapy in the treatment of lung cancer.

Comparing them to well-known medications as cisplatin, however, requires thorough mechanistic research and clinical assessments to verify their safety and therapeutic potential (Ahmed *et al.*, 2022a).

### The effect of leafy extracts versus the cisplatin on oxidative stress and antioxidant status of lung cancer cells

**Oxidative stress markers:** The study examined how cisplatin and green leafy extracts affected the oxidative stress indicators in A549 lung cancer cells, Figs. 4-6. According to the results, all green leafy extracts raised levels of ROS, NO, and MDA when compared to the control, suggesting increased oxidative stress. Neem extract was the extract that increased the most, but it was still less than cisplatin, which increased levels of ROS (263.25%), NO (23.57  $\mu$ M), and MDA (8.14 nmol/mg). This implies that although cisplatin has substantial pro-oxidant actions that contribute to its cytotoxicity, some plant extracts, such as neem, also markedly increase oxidative stress, which may cause cancer cells to undergo apoptosis. Their potential as alternative or adjunctive anticancer treatments with potentially less side effects than cisplatin (Lee *et al.*, 2017; Mokhtari *et al.*, 2023) which was supported by the increased ROS (Eftekhari *et al.*, 2022). The fact that there are changes in oxidative stress and antioxidant markers is in agreement with earlier studies which have shown that neem leaf extract is able to influence oxidative and inflammatory reactions in models relating to the lungs. Lee *et al.* [24] found that neem leaf extract reduced pulmonary inflammation caused by cigarette smoke and LPS, thus supporting the idea that it might play a role in regulating oxidative and inflammatory processes. Likewise, previous research has shown that altering apoptotic and stress-related pathways can increase the anticancer effects of cisplatin in lung cancer cells, for example the way saffron enhances cisplatin-induced apoptosis (Mokhtari *et al.*, 2023) and the synergistic inhibition of lung cancer via caspase-3 and NF- $\kappa$ B signalling (Peng *et al.*, 2025). In the current study, the more marked changes in oxidative stress and antioxidant defences, especially those seen with neem and thyme, indicate that these extracts might be responsible for their anticancer activity by affecting the cellular redox balance; yet further investigation is needed to ascertain any direct molecular interactions with cisplatin-related pathways. However, cisplatin-induced oxidative stress is frequently linked to toxicity in healthy tissues, while plant extracts may provide a more balanced profile by killing cancer cells while leaving healthy cells unharmed (Ahmed *et al.*, 2022a; Di *et al.*, 2025). Overall, our results demonstrate the potential of green leafy extracts in the treatment of lung cancer through processes involving oxidative stress and apoptosis (Peng *et al.*, 2025).

### Antioxidant defense system

**Non-enzymatic antioxidants:** The study evaluated how cisplatin and green leafy extracts affected the amount of thiol in A549 lung cancer cells. It found that all treatments significantly decreased thiol levels when compared to the untreated group, Fig. 7. Among the plant extracts, neem extract produced the most noticeable drop in thiol content

40.36%. Cisplatin came in second, at 36.25%, suggesting significant oxidative stress and cellular antioxidant depletion. Thiol levels were also significantly reduced to about 55% by other extracts, including thyme and rosemary, indicating that they may be able to upset redox balance and encourage apoptosis via oxidative processes. Thiol group depletion, which is essential for preserving cellular redox equilibrium and preventing oxidative damage, is consistent with higher ROS and MDA detected in earlier tests, confirming the pro-oxidant and cytotoxic effects (Bahri *et al.*, 2017). These results suggest that green leafy extracts cause oxidative stress-mediated apoptosis in a manner comparable to that of cisplatin, but they might have a distinct toxicity profile, which could lessen chemotherapy side effects (Peng *et al.*, 2025). Overall, by focusing on redox-sensitive pathways complicated in the survival and death of tumor cells, the data demonstrated the potentials of these natural extracts as supplemental or alternative medicines in lung cancer therapy.

**Table 1. The effect of leafy extracts versus the cisplatin on enzymatic antioxidants.**

|           | GPx activity (%)              | SOD activity (%)              | CAT activity (%)              |
|-----------|-------------------------------|-------------------------------|-------------------------------|
| Control   | 100 $\pm$ 2.15 <sup>a</sup>   | 100 $\pm$ 2.12 <sup>a</sup>   | 100 $\pm$ 2.17 <sup>a</sup>   |
| Mint      | 81.23 $\pm$ 2.17 <sup>b</sup> | 86.21 $\pm$ 1.89 <sup>b</sup> | 81.36 $\pm$ 2.19 <sup>b</sup> |
| Sidr      | 72.3 $\pm$ 2.12 <sup>c</sup>  | 77.2 $\pm$ 2.08 <sup>c</sup>  | 71.25 $\pm$ 1.18 <sup>c</sup> |
| Thyme     | 56.13 $\pm$ 1.92 <sup>d</sup> | 62.4 $\pm$ 2.14 <sup>d</sup>  | 54.36 $\pm$ 2.13 <sup>d</sup> |
| Rosemary  | 53.4 $\pm$ 2.14 <sup>d</sup>  | 56.23 $\pm$ 1.82 <sup>e</sup> | 51.63 $\pm$ 2.16 <sup>d</sup> |
| Basil     | 79.25 $\pm$ 1.93 <sup>b</sup> | 80.32 $\pm$ 1.91 <sup>c</sup> | 36.06 $\pm$ 2.08 <sup>f</sup> |
| Neem      | 44.39 $\pm$ 2.25 <sup>e</sup> | 45.36 $\pm$ 2.12 <sup>f</sup> | 41.12 $\pm$ 1.92 <sup>e</sup> |
| Cisplatin | 35.2 $\pm$ 2.14 <sup>f</sup>  | 41.36 $\pm$ 2.18 <sup>e</sup> | 34.23 $\pm$ 1.89 <sup>f</sup> |

Significant differences ( $p < 0.05$ ) between experimental groups are shown in the data as mean  $\pm$  standard deviation (SD)

**Enzymatic antioxidants:** The study examined how different green leafy extracts affected the activity of the antioxidant enzymes GPx, SOD, and CAT in A549 lung cancer cells in comparison to cisplatin. According to the results, all of the plant extracts that were examined decreased these enzyme activities in comparison to the control; the most notable declines were caused by neem and cisplatin treatments (GPx: 44.39% and 35.2%, respectively; SOD: 45.36% and 41.36%; CAT: 43.04% and 36.12%), Table 1. Mint and basil extracts demonstrated moderate effects, while basil had an exceptionally low CAT activity (36.06%). Thyme and rosemary extracts also significantly inhibited enzyme activities, albeit to a lesser degree than neem or cisplatin. These decreases in antioxidant enzyme activity point to elevated oxidative stress, which could be a factor in the extracts' and cisplatin's ability to induce apoptosis in A549 cells (Almeer *et al.*, 2019; Lee *et al.*, 2025). According to the comparative data, some green leafy extracts, especially neem, showed cytotoxic effects that were comparable to those of cisplatin. This could be due to mechanisms involving oxidative stress and apoptosis pathways (Lee *et al.*, 2025), which were similar to those found in natural compounds that increase the effectiveness of cisplatin in lung cancer cells (Bahri *et al.*, 2017; Shaito *et al.*, 2024; Nam *et al.*, 2025). By regulating oxidative stress and apoptotic responses, this supports the possible use of specific plant extracts as supplemental medicines to treat lung cancer cells.

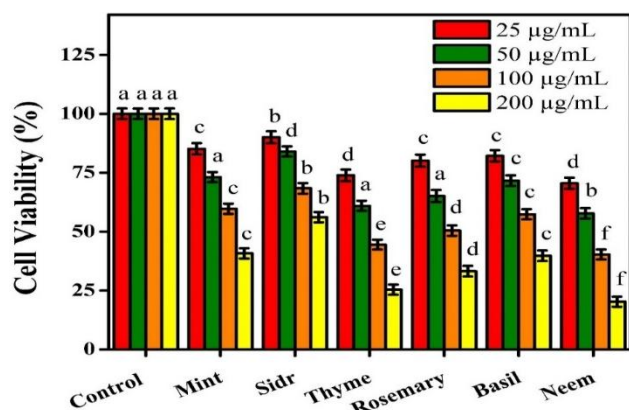


Fig. 3. Cell viability counts among green leafy in A549 lung cells.

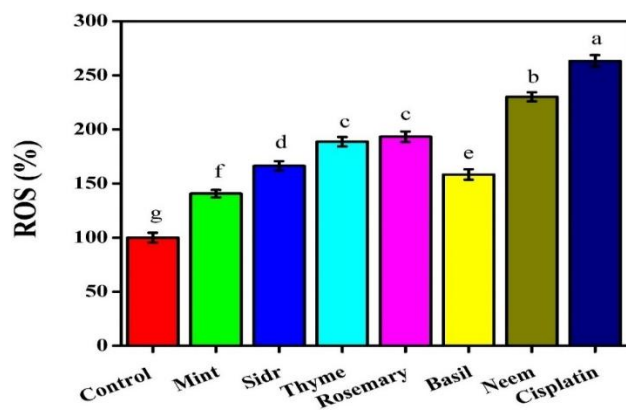


Fig. 4. ROS levels among green leafy in A549 lung cells.

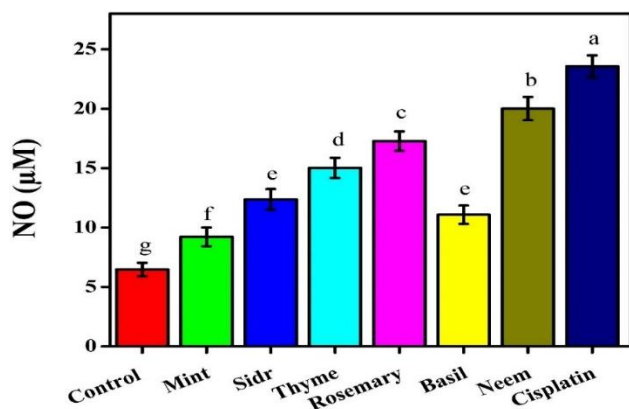


Fig. 5. NO levels among green leafy in A549 lung cells.

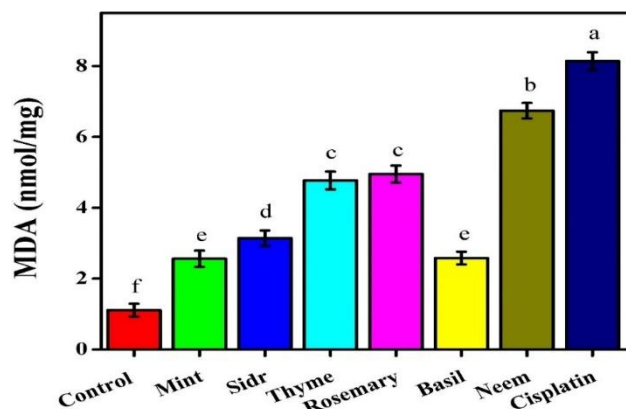


Fig. 6. MDA levels among green leafy in A549 lung cells.

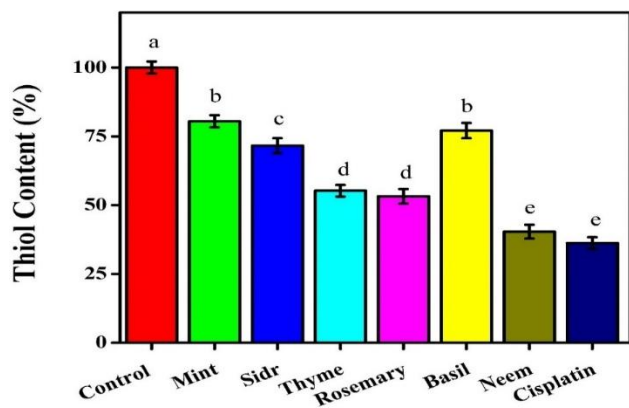


Fig. 7. Thiol levels among green leafy in A549 lung cells.

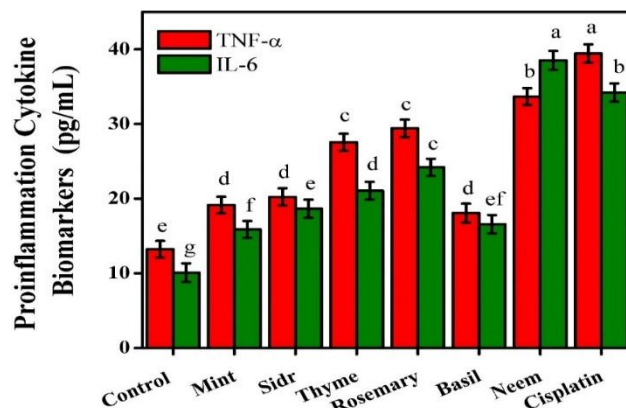


Fig. 8. Inflammatory cytokine levels among green leafy in A549 lung cells.

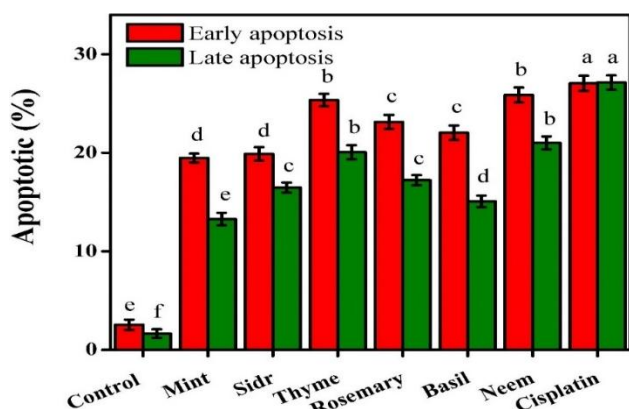


Fig. 9. Apoptotic cell levels among green leafy in A549 lung cells.

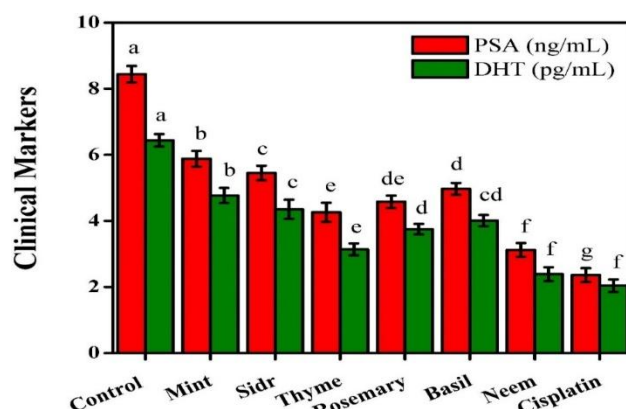


Fig. 10. PSA and DHT levels among green leafy in A549 lung cells.

**The effect of leafy extracts versus the cisplatin on inflammatory cytokines of lung cancer cells:** The pro-inflammatory cytokines TNF- $\alpha$  and IL-6 were measured in the study. Neem exhibited the highest elevation among the plant extracts (TNF- $\alpha$ : 33.67 pg/mL; IL-6: 38.51 pg/mL), however all plant extracts raised TNF- $\alpha$  and IL-6 levels in comparison to the control. As seen in Fig. 8, neem generated a higher level of IL-6 (38.51 vs. 34.21 pg/mL) but a lower level of TNF- $\alpha$  (33.67 vs. 39.44 pg/mL) when compared to cisplatin, Fig. 8. Mint, sidr, and basil caused mild elevations in cytokine levels, whereas rosemary and thyme levels, indicating a severe inflammatory response. These increased levels of cytokines indicated that cisplatin and certain plant extracts might encourage apoptosis, activating inflammatory pathways (Almeer *et al.*, 2019; Ashraf *et al.*, 2025). According to the research, certain green leafy extracts, particularly neem, may imitate and intensify the pro-apoptotic effects of cisplatin by modifying inflammatory mediators associated with cancer cell death (Lee *et al.*, 2017). This demonstrated how these natural extracts could be used as supplemental treatments for lung cancer by affecting both oxidative stress and inflammatory reactions (Lee *et al.*, 2025; Tong *et al.*, 2025).

#### **The effect of leafy extracts versus the cisplatin on apoptosis and mitochondrial pathway markers of lung cancer cells**

**Apoptotic cell percentage:** These extracts stimulate programmed cell death processes, as detected by the increased populations of Annexin-V+/PI- (early apoptosis) and Annexin-V+/PI+ (late apoptosis) (Al Shahrani *et al.*, 2021; Ashraf *et al.*, 2025). In contrast to the control group, which displayed modest apoptosis rates (early apoptosis 2.54%, late apoptosis 1.65%), the results demonstrated that treatment with green leafy extracts dramatically boosted both early and late apoptosis in A549 lung cancer cells, Fig. 9. Neem and Thyme produced the highest levels of early apoptosis (25.87% and 25.36%, respectively) and late apoptosis (21.01% and 20.06%, respectively) among the extracts; these effects were quite similar to those of cisplatin (early apoptosis 27.05%, late apoptosis 27.14%). While slightly less so than neem and thyme, other extracts as rosemary, basil, mint, and sidr also significantly increased apoptotic rates. According to these results, a number of green leafy extracts can cause A549 cells to undergo apoptosis almost as successfully as

cisplatin (Almeer *et al.*, 2019; Muthu *et al.*, 2025), a common chemotherapeutic drug with strong cytotoxicity (Molina-Romo *et al.*, 2018; Hamza *et al.*, 2023). This is consistent with recent studies that demonstrate the capacity of natural substances to modify apoptotic signaling in lung cancer cells (Xu *et al.*, 2025). Although their precise molecular pathways need to be further clarified, the data generally suggest the potential of several green leafy extracts as complementary and alternative medicines to cisplatin for triggering apoptosis in the treatment of lung cancer (Wu *et al.*, 2014; Liao *et al.*, 2020).

**Apoptotic proteins:** The green leafy extract treatment significantly reduced the expression of the anti-apoptotic proteins BCL-2 and BCL-XL in A549 lung cancer cells compared to the control group, which maintained 100% expression of both proteins. Neem extract therapy resulted in the largest reduction in BCL-2 (42.09%) and BCL-XL (46.98%) expression. This was closely followed by cisplatin, which reduced BCL-2 and BCL-XL expression to 37.22% and 41.22%, respectively. Consequently, compared to the control levels (0.84 and 0.88 U/mg protein, respectively), all treatments increased the activities of caspase-9 and caspase-3, two crucial enzymes linked to the intrinsic apoptotic pathway; cisplatin induced the highest activities (3.25 and 3.89 U/mg protein, respectively). The results indicated that the apoptotic activity of the neem and thyme mainly involved the intrinsic mitochondrial pathway, which was evident by the downregulation of BCL-2/BCL-XL and the upregulation of caspase-9 and caspase-3, and that the increase in TNF- $\alpha$  and IL-6 could be related to the associated inflammatory signaling. Extracts of rosemary and thyme similarly markedly decreased the expression of BCL-2 family proteins and boosted caspase activity, suggesting that cisplatin-like but less potent activation of mitochondrial apoptosis pathways occurred (Kamalia & Tunjung, 2023; Curtis *et al.*, 2024). These apoptotic indicators showed mild effects from extracts of mint, sidr, and basil, indicating a compound-specific or dose-dependent impact on apoptosis induction (Saeed *et al.*, 2025). Together, these findings implied that green leafy extracts can successfully induce apoptosis in A549 cells by inhibiting anti-apoptotic BCL-2 proteins and triggering caspase cascades. Neem extract exhibits potential similar to that of cisplatin, suggesting their potential use as complementary agents in lung cancer therapy (Farmani *et al.*, 2016; Sari *et al.*, 2025; Alaboudi *et al.*, 2025) (Table 2).

**Table 2. The effect of leafy extracts versus the cisplatin on apoptotic proteins.**

|           | BCL-2 Expression (%)          | BCL-XL Expression (%)         | Caspase-9 (U/mg protein)      | Caspase-3 (U/mg protein)     |
|-----------|-------------------------------|-------------------------------|-------------------------------|------------------------------|
| Control   | 100 $\pm$ 1.98 <sup>a</sup>   | 100 $\pm$ 2.02 <sup>a</sup>   | 0.84 $\pm$ 0.11 <sup>g</sup>  | 0.88 $\pm$ 0.11 <sup>f</sup> |
| Mint      | 70.33 $\pm$ 1.79 <sup>b</sup> | 74.95 $\pm$ 1.94 <sup>b</sup> | 1.55 $\pm$ 0.19 <sup>f</sup>  | 1.88 $\pm$ 0.15 <sup>e</sup> |
| Sidr      | 65.11 $\pm$ 2.12 <sup>c</sup> | 70.04 $\pm$ 2.09 <sup>c</sup> | 1.82 $\pm$ 0.18 <sup>e</sup>  | 2.18 $\pm$ 0.14 <sup>d</sup> |
| Thyme     | 51.09 $\pm$ 2.09 <sup>f</sup> | 56.21 $\pm$ 2.12 <sup>e</sup> | 2.47 $\pm$ 0.15 <sup>c</sup>  | 2.87 $\pm$ 0.17 <sup>c</sup> |
| Rosemary  | 55.68 $\pm$ 2.14 <sup>c</sup> | 59.36 $\pm$ 1.91 <sup>e</sup> | 2.23 $\pm$ 0.12 <sup>cd</sup> | 2.66 $\pm$ 0.16 <sup>c</sup> |
| Basil     | 61.37 $\pm$ 2.05 <sup>d</sup> | 64.17 $\pm$ 2.11 <sup>d</sup> | 2.11 $\pm$ 0.11 <sup>d</sup>  | 2.41 $\pm$ 0.15 <sup>d</sup> |
| Neem      | 42.09 $\pm$ 2.16 <sup>g</sup> | 46.98 $\pm$ 2.21 <sup>f</sup> | 2.99 $\pm$ 0.14 <sup>b</sup>  | 3.48 $\pm$ 0.12 <sup>b</sup> |
| Cisplatin | 37.22 $\pm$ 1.91 <sup>h</sup> | 41.22 $\pm$ 2.14 <sup>g</sup> | 3.25 $\pm$ 0.13 <sup>a</sup>  | 3.89 $\pm$ 0.13 <sup>a</sup> |

Significant differences ( $p < 0.05$ ) between experimental groups are shown in the data as mean  $\pm$  standard deviation (SD)

### The effect of leafy extracts versus the cisplatin on clinical markers of lung cancer cells:

The study examined how different green leafy extracts and cisplatin affected the A549 lung cancer cell line's cell survival, apoptosis, and PSA and DHT levels. All green leafy extracts decreased PSA and DHT levels when compared to the control, according to the results, Fig. 10. The treatments with neem and cisplatin produced the biggest reductions, suggesting stronger anti-proliferative actions. Previous researches have demonstrated that plant extracts can alter apoptotic pathways and inhibit proliferation in lung cancer cells, which is consistent with the extracts' capacity to induce apoptosis and reduce PSA and DHT (Lee *et al.*, 2011; Wu *et al.*, 2014). Green leafy extracts presented a prospective substitute with the possibility for less side effects in contrast to cisplatin, which demonstrated strong cytotoxicity but also higher toxicity concerns. These findings are in line with research demonstrating that by triggering apoptosis and altering hormones, natural compounds can halt the growth of lung cancer cells (Nasser *et al.*, 2022), suggesting that they could be used in addition to or instead of traditional chemotherapy. The observed anticancer properties could be attributed to the various phytochemical classes found in these plants, including flavonoids, terpenoids, and alkaloids, which have been reported to have effects on oxidative stress, inflammatory signaling, and apoptotic pathways. The results were in line with the recent research indicating that leaf extract of *Azadirachta indica* and its major component, azalene exhibit dose-dependent cytotoxic and apoptosis inducing effect on A549 lung cancer cells (Toson *et al.*, 2026), and thymus species and their major component thymol showed antiproliferative and mitochondria-mediated apoptosis inducing activity in lung cancer models.

### Conclusion

The ethanolic extracts of the leafy green plants showed antiproliferative activities in A549 lung cancer cells, particularly *Azadirachta indica* (neem) and *Thymus vulgaris* (thyme) plants. The results indicate activation of the intrinsic pathway of mitochondrial apoptosis since BCL-2 and BCL-XL expression were down-regulated and the expression of caspase-9 was up-regulated.

**Study Limitation:** The specific compounds underlying the observed anticancer effects cannot be definitively assigned as several bioactive phytochemicals have been reported in the plants investigated, but not in the current study, which did not involve HPLC or LC-MS/MS analysis. Further research involving chromatographic profiling and mass spectrometry coupled with bioactivity-based fractionation could be useful to identify and quantify the active compounds and correlate them directly with their activity. Neem and thyme might have the potential to be used as an adjunct to cisplatin because it can enhance the anticancer activity and may even reduce the dose of cisplatin required, but more research is needed to explore how they work synergistically and to determine if they can reduce the toxicity associated with cisplatin.

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