Fig. 2 — Dose-dependent free radical scavenging potential of *Solanum lycopersicum* L.leaf extract (SLLE) determined by (A) DPPH; and (B) ABTS assays. By using Analysis of Variance, the data were examined for statistical significance. By using Analysis of Variance, the data were examined for statistical significance. The statistical significance between the test samples was determined using Tukey's test, and a *P*-value of 0.05 or lower was regarded as significant. Bar graphs with different alphabets show that statistical significance in the particular study group

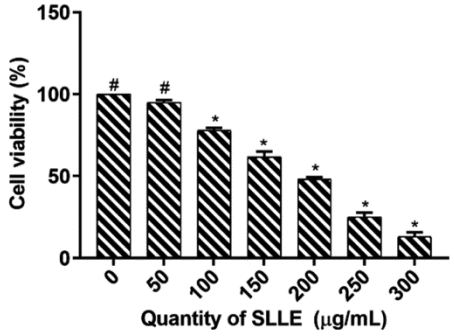

Fig. 3 — Dose-dependent anticancer effect of *Solanum lycopersicum* L. leaf extract (SLLE) determined by MTT assay. By using Analysis of Variance, the data were examined for statistical significance. The statistical significance of the control and test samples was determined using Dunnett's test, and a *P*-value of 0.05 or lower was regarded as significant. The asterisk (#) indicates that there is no significant difference between the test sample and the control sample. While the asterisk (*) denotes the significance of the test sample relative to the control

Fig. 4 — Dose-dependent effect of *Solanum lycopersicum* L.leaf extract (SLLE) on the release of lactate dehydrogenase (LDH). By using analysis of variance, the data were examined for statistical significance. The statistical significance of the control and test samples was determined using Dunnett's test, and a *P*-value of 0.05 or lower was regarded as significant. The asterisk (#) indicates that there is no significant difference between the test sample and the control sample. While the asterisk (*) denotes the significance of the test sample relative to the control

viable cells[30]. 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[31,32]. 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[33]. 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

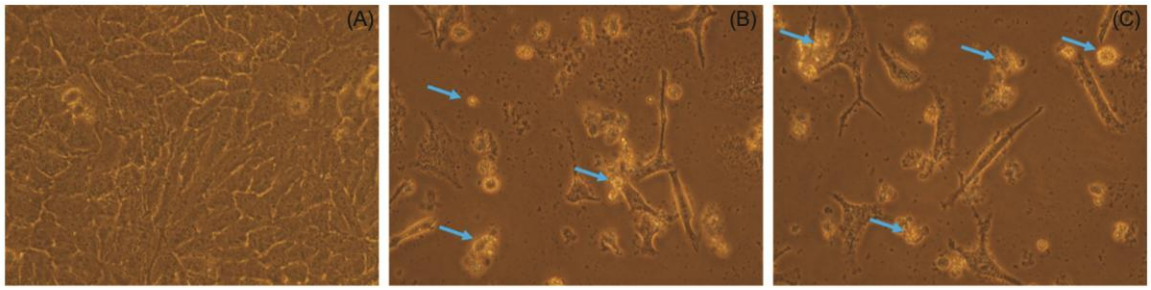

Fig. 5 — Bright-field inverted microscope images depicting the detrimental impact of cisplatin and *Solanum lycopersicum* L.leaf extract (SLLE) on the micro-morphology of A549 cancer cells. (A) Control cells. (B) Cells treated with SLLE at its IC_{50} value of 190.41 ± 4.77 µg/mL. (C) Cells treated with cisplatin at its IC_{50} value of 5.20 ± 0.09 µM. The arrow marks show detrimental damage to cells. The depictions were displayed with 400x magnification

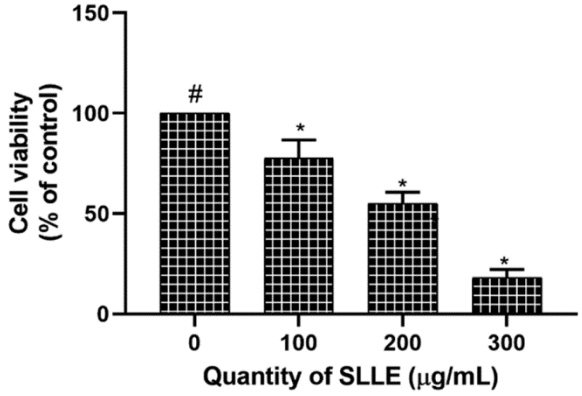

Fig. 6 — Dose-dependent effect of *Solanum lycopersicum* L.leaf extract (SLLE) on cell viability determined by live/dead dual staining analysis. By using Analysis of Variance, the data were examined for statistical significance. The statistical significance of the control and test samples was determined using Dunnett's test, and a *P*-value of 0.05 or lower was regarded as significant. The asterisk (#) indicates that there is no significant difference between the test sample and the control sample. While the asterisk (*) denotes the significance of the test sample relative to the control

with MTT and LDH assay.

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[17]. The assay showed that SLLE has dose-dependently reduced the viability of cells (Fig. 6). The effect of SLLE on the viability of the cells was shown in (Fig. 7). The control cells were green indicating that cells were viable. Whereas, cells treated with IC_{50} levels of SLLE (190.41 ± 4.77 µg/mL) and cisplatin (5.20 ± 0.09 µM) have a low level of green cells (live cells) and noticed a high level of dead cells (red color) compared to control 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[34,35]. In the present study, SLLE has dose-dependently escalated the caspase-3 levels (Fig. 8). 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 is what causes them to have positive health impacts. The primary mechanisms by which plant secondary compounds exerttheir 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[36]. In our study, SLLE has shown potential anticancer due to the attendance of phenols, flavonoids, terpenoids, and alkaloids.

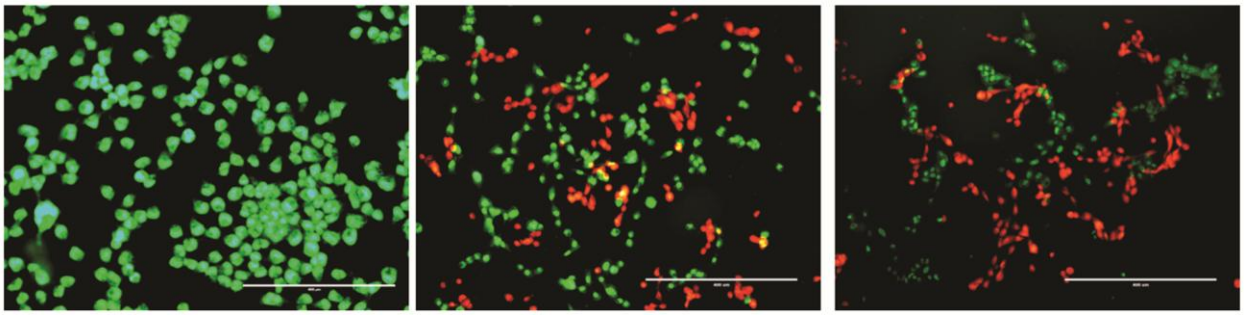

Fig. 7 — Fluorescent microscopic images depicting the impact of cisplatin and *Solanum lycopersicum* L.leaf extract (SLLE) on the viability of A549 cancer cells. The green color represents live cells and the red color represents dead cells. (A) Control cells;(B) Cells treated with SLLE at its IC_{50} value of 190.41 ± 4.77 µg/mL; and (C) Cells treated with cisplatin at its IC_{50} value of 5.20 ± 0.09 µM. Scale bar = 400 µM

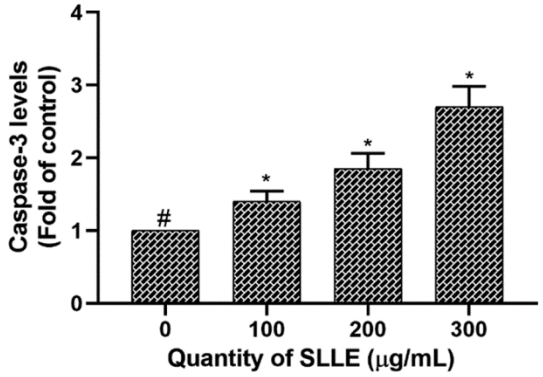

Fig. 8 — Dose-dependent effect of *Solanum lycopersicum* L. leaf extract (SLLE) on caspase-3 levels. By using Analysis of Variance, the data were examined for statistical significance. The statistical significance of the control and test samples was determined using Dunnett's test, and a *P*-value of 0.05 or lower was regarded as significant. The asterisk (#) indicates that there is no significant difference between the test sample and the control sample. While the asterisk (*) denotes the significance of the test sample relative to the control

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.

Acknowledgement

The authors were thankful to Acharya Nagarjuna University for providing the facility.

Conflict of interest

All authors declare no conflict of interest.

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