Lung cancer is a disease with high morbidity and high mortality rates. As a result, it is often associated with five year survival rate of 17%. A better understanding of the prevalent disease and improvements in the treatment are greatly needed for better diagnosis and therapeutic design for cancer treatments. Nowadays, medicinal plants play an important role in the treatment of various diseases including cancers. The plant *Luffa acutangula* L. (*Roxb)* (Cucurbitaceae) is one of the common climber found in South India. It possesses various pharmacological activities; the fruits are being used in traditional medicine under the name of jalini for various medicinal purposes. The aim of the study is focused on the bioassay guided isolation, structure elucidation and anticancer activity of bioactive compound of aerial parts of *L. acutangula*.

**Chapter-I** focused on the preliminary phytochemical investigation, *in vitro* antioxidant and anticancer activities of the crude ethanol extract of *L. acutangula*.

- The preliminary phytochemical investigation of various extracts *viz.*, petroleum ether (9.0%), ethyl acetate (13.0%), ethanol (22.5%) and water (13.5%) showed the presence of various secondary metabolites such as alkaloids, terpenoids, flavonoids, saponins and steroids.
- The ethanol extract showed the presence of phenol content (67.23±4.455), flavonoid (45.73±0.029), tannin content (28.03±0.288) and alkaloid content (12.07±0.310).
- The antioxidant properties of the ethanol extract was evaluated using various *in vitro* models which suggested that the maximum radical scavenging activity was found at 2.5 μg/ml.
- The antiproliferative activity of ethanol extract revealed a gradual decrease in cell viability of NCI-H460 cells in a dose dependent manner and exhibited 50% of cell death at the concentration of 25 μg/ml after 24 h treatment.
• In addition, the apoptotic activity of the ethanol extract showed significantly increased ROS generation in cancer cells at 25 µg/ml for 24 h incubation. It can alter the mitochondrial depolarization in NCI-H460 cells at 25 µg/ml for 24 h incubation.

• The morphological characteristics of untreated cells showed pale orange colour whereas, L. acutangula crude extract treated cells (MMP altered cells) exhibit only green fluorescence.

• The dual staining result shows that the number of apoptotic cells were increased in the extract treated group. The above results indicate that the plant extract shows potent antiproliferative activity against NCI-H460 cells.

• Growth inhibition and ROS generation by L. acutangula ethanol extracts in NCI-H460 cells indicate that ROS production probably causes apoptotic cell-death via the mitochondrial pathway.

With this background, the Chapter-II focused on the isolation and purification of bioactive compound from an ethanol extract of L. acutangula through bioassay-guided isolation approach.

• Five major fractions were subjected to MTT assay. Among these fractions, the LA/FII effectively decreased the growth of cancer cells with IC_{50} value of 10 µg/ml concentration.

• MMP analysis revealed the loss of cell membrane integrity (green fluorescence) in LA/FII treated cells compared to control cells (pale orange color). This result signifies that the LA/FII treated NCI-H460 cells underwent complete death through the depolarization of their MMP at 20 µg/ml concentration.

• Also, LA/FII significantly increased intracellular ROS level which strongly revealed that most of the cells underwent oxidative stress by which apoptosis
The apoptogenic activity of fraction LA/FII was confirmed by cell shrinkage, membrane blebbing and formation of apoptotic bodies, which are characterized by intense orange-red fluorescence and reduced green fluorescence and these observations indicate the presence of apoptotic cells.

Based on the bioassay, the active fraction LA/FII was rechromatographed to afford bioactive compound (70 mg) and purity of the compound was confirmed by TLC.

The structural elucidation of the bioactive compound was analysed by UV, IR, ESI-MS, \(^1\)H and \(^{13}\)C NMR spectra and the molecular formula of the bioactive compound was determined as C\(_{15}\)H\(_{10}\)O\(_{4}\).

Based on the spectral characterization, the bioactive compound was named as 1, 8-dihydroxy-4-methylanthracene-9, 10-dione (DHMA), an anthraquinone derivative.

**Chapter-III** deals with anticancer activity of bioactive compound from *L. acutangula* and its impact on pro and anti-apoptotic proteins (p53, p21, Bax, caspase-3 and NF-\(\kappa\)B) in cancer cell line.

- The DHMA exhibited significant inhibitory effect on the proliferation of NCI-H460 cells with IC\(_{50}\) value of 50 \(\mu\)g/ml concentration after 24 h treatment.
- The DHMA remarkably increase the ROS-associated fluorescence intensity when compared to control and this showed significant reduction in cell viability associated with apoptotic effect in NCI-H460 cell line.
- AO/EtBr method showed typical morphological changes in DHMA treated cells, such as chromatin condensation, apoptotic bodies and chromatin marginalization which demonstrates that cell death occurred primarily via
apoptosis.

- DNA extracted after 24 h treatment with DHMA revealed a ladder corresponding to DNA fragmentation, which showed an indication of apoptotic cell death induction, substantiated by presence of apoptotic bodies in dual staining method.

- The expression of p53, Bax and GADD45A were markedly increased and ATM expression was down-regulated at transcription level in treated cells. These results indicate that NCI-H460 cells have undergone cell cycle arrest and DNA damage through apoptotic process after DHMA treatment.

- In this study, treatment of DHMA on NCI-H460 cells caused a significant increased of p53, p21, bax and caspase-3 protein expression and also suppressed the expression of NF-kB, which implies that DHMA may promoted apoptosis via the up-regulation of p53 and down-regulation of NF-kB.

- The molecular docking studies suggested that the OH groups of DHMA involved in the formation of hydrogen bond with carbonyl group of Trp23 and Phe55 of MDM2 with docking score of -5.662 kcal/mol.

- A 20 ns MD simulation of MDM2-p53-DHMA complex showed the stability of the system, which is evident from the minimum RMSD and low RMSF values and there is no much fluctuation in the active site residues such as Trp53, Phe55 and Lys24.

- The docking and dynamics results indicate that the DHMA form a strong hydrogen bond interaction with the hydrophobic residue Trp23, a residue which plays a leading role in MDM2-p53 interaction.

- The interaction of bioactive compound with Trp23 of p53 was responsible for the
disruption of MDM2-p53 interaction and it was concluded that DHMA have the efficacy to activate the expression of apoptosis related proteins.

**Chapter-IV** focused to investigate the molecular interaction of DHMA with DNA binding domain of NF-κB monomer and NF-κB-DNA (1NFK).

- Nuclear factor kappa B (NF-κB), transcription factor plays a crucial role in the regulation of various physiological processes such as differentiation, cell proliferation and apoptosis.

- The experimental results (Chapter-III) evidently suggested that DHMA effectively induces apoptosis through suppression of NF-κB protein. Based on experimental evidence, the binding affinity of DHMA with NF-κB p50 (monomer) and NF-κB-DNA was investigated using three different docking protocols (XP, IFD and QPLD).

- In addition, the similar scaffold compounds of DHMA was screened from natural product database and as an outcome, the compound THA with 80% similarity in its scaffold to that of DHMA was computed.

- The molecular docking results clearly suggested that binding pose of DHMA and THA share a similar binding mode in the DNA binding region of NF-κB. However, comparing the binding free energy and number of interaction, it was noticed that the pose obtained from the IFD shows more interaction with NF-κB as well as NF-κB-DNA complex than XP and QPLD.

- Both DHMA and THA exhibited hydrogen bond interaction with active site residues Tyr57, His114 and Asp239, which are important for DNA binding to the NF-κB monomer (p50). The hydroxyl group plays an important role in protein-ligand interaction. While in NF-κB-DNA complex, both compounds interacts with phosphate group of DG-4 and DG-5 via hydrogen bond interaction
Further, the rescoring of both DHMA and THA complexes with NF-κB monomer (p50) and NF-κB-DNA shows that DHMA has acquired relatively high binding free energy with NF-κB (-43.002 kcal/mol) and NF-κB-DNA (-34.769 kcal/mol). This result clearly indicates that the hydroxyl groups (OH) of most stable conformer of DHMA form strong hydrogen bond interactions with DNA and therefore suggested that the binding is stronger with NF-kB-DNA complex than NF-kB monomer.

The molecular dynamics studies emphasized that DHMA and THA complexes with NF-kB monomer and NF-kB-DNA were more stable during the 10 ns simulation period compared to apo-protein. The DHMA also maintained stable binding conformation throughout the simulation with DNA and these results are corroborated with molecular docking studies.

The reactivity of the compounds evaluated using DFT methods suggested that the energy gap value of DHMA and THA is more or less similar with low HOMO-LUMO energy gap, which determines the stability of molecules.

The ADME results suggested that all the pharmacokinetic properties of both compounds DHMA and THA were in acceptable range.

Overall the above findings suggest that naturally occurring 1, 8-dihydroxy-4-methylantracene-9, 10-dione (DHMA) is a potent inhibitor of NF-κB activation and may act as a promising agent for anticancer drug development.