



Research Article

**APOPTOTIC PROPERTIES OF LEAF EXTRACTS OF *SIMAROUBA GLAUCA* AGAINST LUNG CANCER- A549 CELLS**

Amirthavarshini.A<sup>1\*</sup>, M.Latharani<sup>2</sup>, S.Mathukumar<sup>3</sup>

\*<sup>1</sup>CRRI, <sup>2</sup>Professor, Dept. of Aruvai Thol Maruthuvam, <sup>3</sup>Principal, Sri Sairam Siddha Medical College and Research Centre, West Tambaram, Chennai, India.

Article info

Article History:

Received: 19-05-2024

Accepted: 15-06-2024

Published: 10-07-2024

KEYWORDS:

Simaroubaglauca, cytotoxic, Apoptotic, Anticancer activity, Antiproliferative, Lung cancer, A549 cells.

ABSTRACT

*Simarouba glauca* belonging to the family of Simaroubaceae and is commonly known as The Paradise tree, Laxmitaru tree (Common name) or *Sorgamaram* (Siddha Name). It has a long history of herbal medicine in many countries. It is a native of South America and its bark decoction is given for dysentery and hence called dysentery bark. The drug contains phytochemicals such as glaucarubin, quassinoids, aianthione, benzoquinone, holacanthone, melianone, simaroubidin, simarolide, sistosterol. **Objectives:** This study is to evaluate apoptotic activity of leaf extracts of *Simarouba glauca* against A549 Lung Cancer Cell line using MTT Assay and determining the % cell viability on increasing concentrations. **Materials and Methods:** Plant sample is collected and leaf Extract is prepared by adding 5gm of dried leaves powder in 50 ml aqueous extract. And to assess cell proliferation and viability MTT cell viability test was performed and apoptotic properties were identified. Aqueous extract of various concentration were used (12.5, 25, 50, 100, 200µg/ml) to confirm the increased apoptotic activity on increasing concentration. **Conclusion:** These findings suggested that *Simarouba glauca* leaf extracts inhibited lung cancer cells in a time- and dose-dependent manner. The leaf extracts of *Simarouba glauca* was found to be nontoxic to alveoli, pneumocytes. It can be concluded that *Simarouba glauca* is an important source of phytochemicals posing efficacy against lung cancer cells.

INTRODUCTION

Lung cancer is one of the most constantly diagnosed cancers and the leading cause of cancer-related deaths worldwide with an estimated 2 million new cases and 1.76 million deaths per time. Lung cancers generally start in the cells lining the bronchi and corridor of the lung similar as the bronchioles or alveoli [10]. Lung cancer includes two main types non-small cell lung cancer and small cell lung cancer. Smoking causes most lung cancers, but non-smokers can also develop lung cancer[2]. About 80 to 85 of lung cancers are (Non-Small Cell Lung Cancer) NSCLC. The main subtypes of NSCLC are adenocarcinoma, scaled cell melanoma, and large cell melanoma and about 10 to 15% of all lung cancers are (Small Cell Lung Cancer)

SCLC and it's occasionally called oat cell cancer. This type of lung cancer tends to grow and spread briskly than Non-small cell lung cancer NSCLC. About 70% of people with SCLC will have cancer that has formerly spread at the time they're diagnosed[8]. Other types include Lung Carcinoid Tumour which regard for 5% of lung tumours, adenoid cystic lymphomas, tubercles, and sarcomas, as well as benign lung tumours similar as hamartomas are rare. Cancer can metastasize from other organs similar as bone, liver, spleen, order to lungs and treatment for cancer from primary point of origin is given. Presently numerous studies have been done to identify phytochemical ingredients, cytotoxicity, medium of action of numerous sauces in experimental and clinical cases. So far, pharmaceutical companies have screened further than 25,000 shops to discover anticancer medicines. It's important to experimentally validate sauces and herbal phrasings so that cancer can be averted, can record lower morbidity and mortality due to tumours, adverse responses due to chemotherapy can be minimised. Medicine discovery and development from factory- grounded

Access this article online	
Quick Response Code	<a href="https://doi.org/10.47070/ijapr.v12i6.3213">https://doi.org/10.47070/ijapr.v12i6.3213</a>
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factors is cost-effective than conventional synthetic emulsion product.

*Simarouba glauca* belonging to the family of Simaroubaceae is an evergreen, small or medium-sized tree with a narrow crown. It generally grows up to 15 metres altitudinous but samples up to 27 metres are recorded. The straight, spherical caddy can be free of branches for over to 9 metres, 30 cm or further in diameter [3].

The plant is called as Lakshmi taru in Sanskrit and Sorgham Maram in Tamil. It contains phytochemicals similar as Quassinoids, glaucarubin, glaucarubinone, glaucarubolone, ailanthinone are proved to be anti-protozoal, anti-amoebic, anti-malarial and poisonous to cancer cell lines. *Simarouba glauca* is traditionally given as decoction for cancer treatment. This study aims to demonstrate apoptotic exertion of waterless excerpt of leaves on mortal non-small-cell lung cancer A549 cells.

## MATERIALS AND METHODS

### Plant

The leaves of *S.glauca* were collected from Periyar University Campus, Salem (Figure 1) and organoleptic characters were confirmed by Botanist. The leaves were processed in the following steps, a) washed with tap water to remove dust and adherent materials and b) then shade dried for 3 days and c) powdered using sterile blender. Coarse powder was made as results may vary due to residues in fine powder (Figure 2). This powder was used for sequential solvent extraction using water (Aqueous extract).

### Cell culture maintenance

A549 (human epithelial lung adenocarcinoma cell line) was obtained from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were maintained in the logarithmic phase of growth in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin. They were maintained at 37°C with 5% CO<sub>2</sub> in 95% air humidified incubator.

### Cytotoxicity assay using MTT assay

The anticancer activity of the sample was tested against A549 cell line by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay which relies on the ability of a mitochondrial dehydrogenase

enzyme from healthy cells to cleave the tetrazolium rings of the pale yellow MTT and form impermeable, dark blue formazan crystals, which accumulate within healthy cells (Mossman, 1983). The cells were seeded in 96-well microplates (1 x 10<sup>6</sup> cells/well) and incubated at 37°C for 48 h in 5% CO<sub>2</sub> incubator and allowed to grow 70-80% confluence. Then the medium was replaced and the cells were treated with different concentrations of sample and incubated for 24 h. The morphological changes of untreated (control) and the treated cells were observed under digital inverted microscope (20X magnification) after 24 h and photographed. The cells were then washed with phosphate-buffer saline (PBS, pH-7.4) and 20 µL of (MTT) solution (5 mg/mL in PBS) was added to each well. The plates were then stand at 37°C in the dark for 2 h. The formazan crystals were dissolved in 100 µL DMSO and the absorbance was read spectrometrically at 570 nm<sup>[9]</sup>. Percentage of cell viability was calculated using the formula,

$$\text{Cell viability(\%)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

## RESULTS AND DISCUSSION

In this study aqueous leaf extracts of *Simarouba glauca* has been demonstrated to have cytotoxicity using MTT Assay against A549 cancer cell lines. The cell culture was treated with different concentrations from 12.5 µg/ml to 200 µg/ml of leaf extracts and control was also maintained and after incubation for 24 hours results were noted. The results showed that leaf extracts of *S.glauca* effectively inhibited the proliferation of A549 cell lines in a dose-dependent manner. Cytotoxicity was directly proportional to the concentration of leaf extract. Cell viability (%) for control and various concentrations 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100µg/ml, 200µg/ml are 100 (Figure 4 a), 92.40 (Figure 4 b), 81.16 (Figure 4 c), 67.62 (Figure 4 d), 43.76 (Figure 4 e), 34.67 (Figure 4 f) respectively. Graph was plotted using the cell viability (%) at y-axis and concentration of sample in x-axis (Figure 3). And in control the cell viability in 100% and no cell death noticed. The morphological changes of untreated (control) and treated cells were observed under digital inverted microscope (20X magnification) after 24 hours it was photographed, we are clearly able to visualize apoptotic changes on increasing concentrations of leaf extracts (Figure 4).



Figure 1



Figure 2

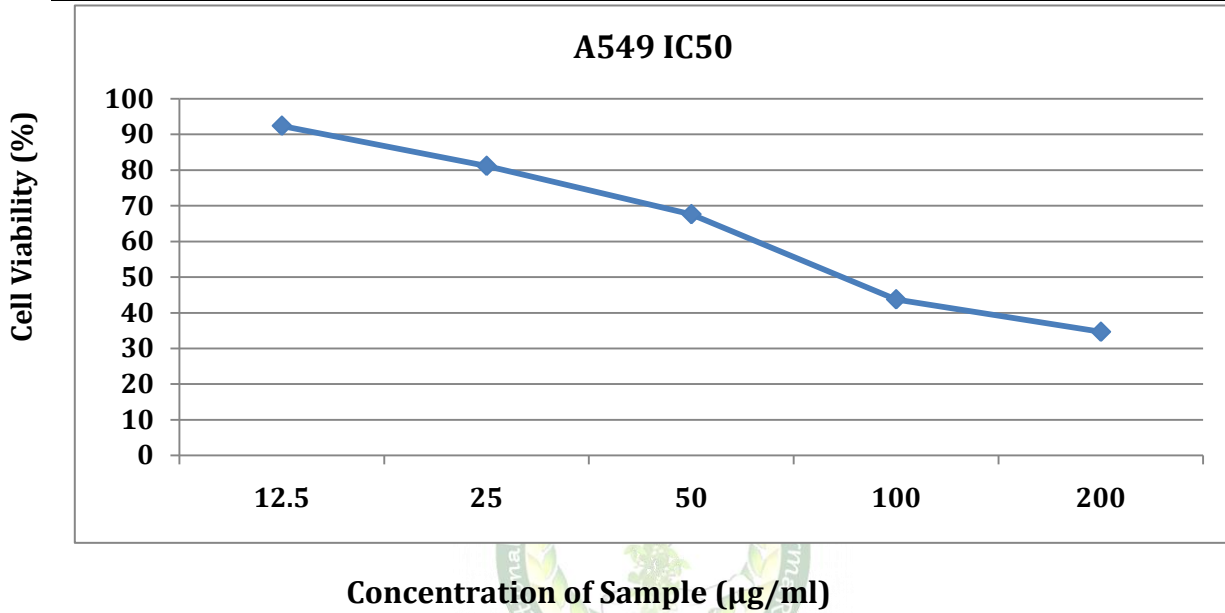


Figure 3: Cytotoxicity of *S. glauca* Aqueous leaf extracts on A549 Lung cancer cell lines

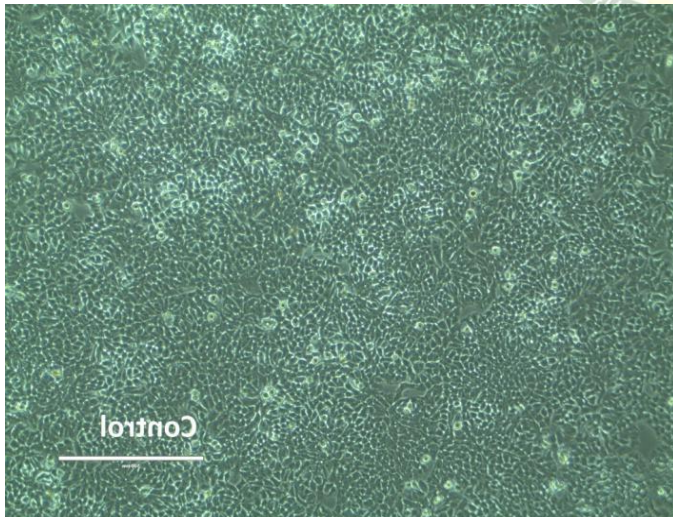


Figure 4 a

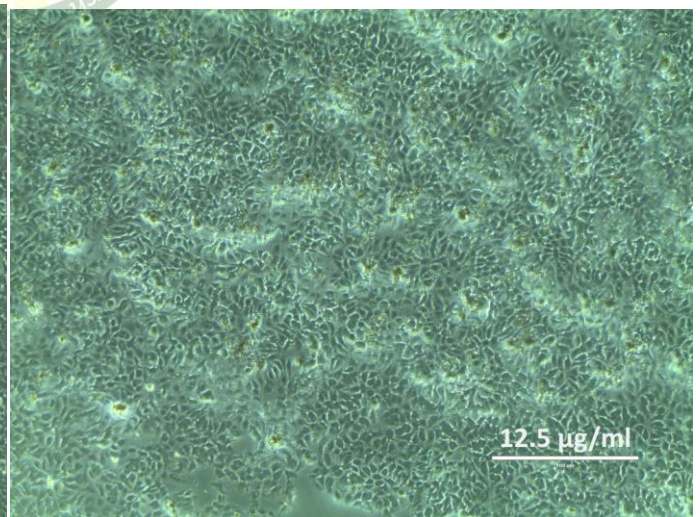
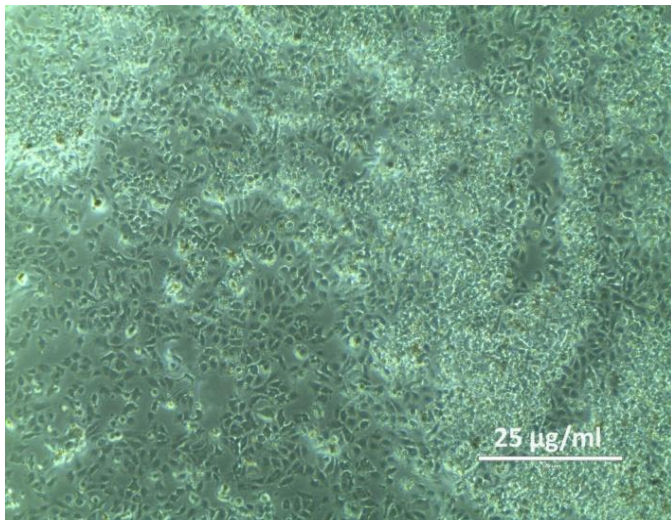
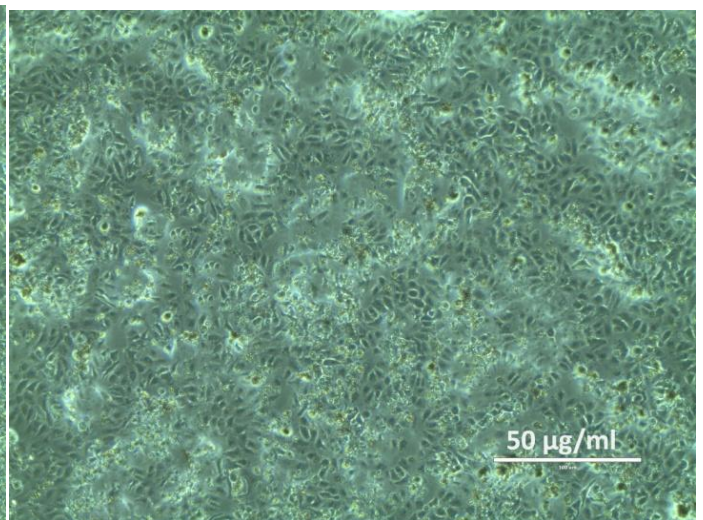


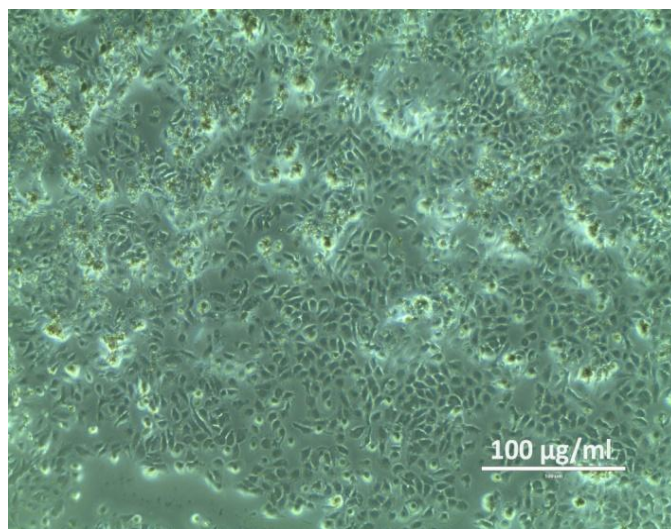
Figure 4 b



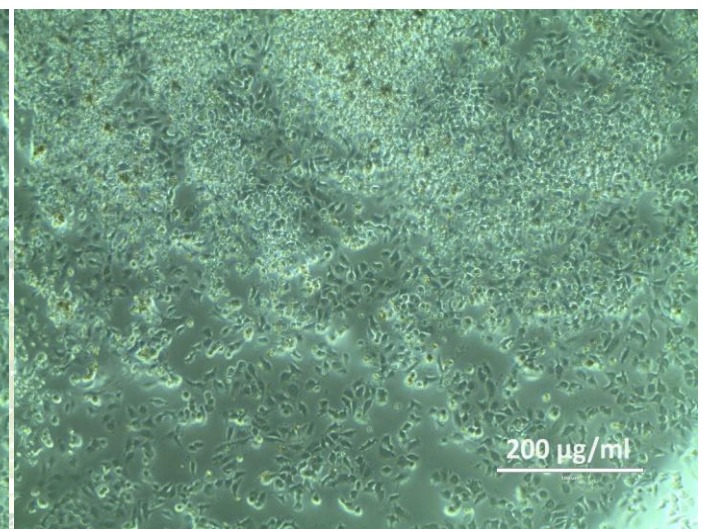
**Figure 4 c**



**Figure 4 d**



**Figure 4 e**



**Figure 4 f**

**Figure 4: Microscopic pictures in various concentrations of *Simarouba glauca* to demonstrate cytotoxicity**

## DISCUSSION

The overall result proved that *S.glauca* exhibit anti-cancer activity and was toxic to A549 lung cancer cell lines. This plant leaves and bark decoction has been given since ancient times for the cure of cancer and hence scientific validation is necessary to prove its safety and efficacy in treating cancer cell lines. Since decoction of the plant based materials are ingested in the treatment, in our study we evaluated the efficacy of aqueous extracts of leaves so that it can be reliable that decoction which is made using water can show cytotoxicity for cancer cell lines. Plant based treatment can be best and reliable for the prevention and treatment of cancer. Since ancient times plants have been used for human resources and it's fascinating that plants can be effective in treating current medical diagnosis and diseases. Many studies have been done to prove apoptotic activities of *S.glauca* against various cancer cell lines. Alcohol extract, Liquid petroleum extracts were also evaluated to prove effective against

Leukemic cancer<sup>[1]</sup>, Bladder cancer<sup>[7]</sup>. Anti-proliferative potential of phytochemical isolated form *S.glauca* was also studied<sup>[5]</sup>. Tricaprion isolated from *S.glauca* inhibits growth of Human Colorectal Carcinoma<sup>[4]</sup>. In-vivo anti cancerous studies are conducted on Ehrlich Ascitis Carcinoma model in mice <sup>[6]</sup>.

## CONCLUSION

The present study substantiated that *Simarouba glauca* possess anti-cancer activity against Non-small-cell-lung cancer, A549 cells. Further research is recommended for the development of the drug.

## ACKNOWLEDGEMENT

I want to extend my thanks for the substantial contribution to Avigen Biotech, Chrompet for Cell Culture Maintenance and Cytotoxicity assay. Dr. Latharani has coordinated and helped to draft the study. I owe my parents a huge debt of gratitude.

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### Cite this article as:

Amirthavarshini.A, M.Latharani, S.Mathukumar. Apoptotic Properties of Leaf Extracts of Simarouba glauca Against Lung Cancer- A549 Cells. International Journal of Ayurveda and Pharma Research. 2024;12(6):30-34.

<https://doi.org/10.47070/ijapr.v12i6.3213>

**Source of support: Nil, Conflict of interest: None Declared**

### \*Address for correspondence

**Dr. Amirthavarshini.A**

CRRI, Sri Sairam Siddha Medical College and Research Centre, Sai leo nagar, West tambaram, Chennai.

Email: [amirhhacgm@gmail.com](mailto:amirhhacgm@gmail.com)

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