



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

In Vitro Evaluation of Anticancer Activity of Sorgamara Ilai Chooranam (Leaves of Simarouba Glauca) Against A549 Cell Line (Human Lung Cancer Cell)

Giftillda Selva Elsee T*¹, Velpandian V¹, Nalina Sarasawathi K¹

1. Post Graduate Department of Gunapadam (Pharmacology), Government

Siddha Medical College, Arumbakkam, Chennai, TN, India.

ABSTRACT

Lung cancer is the leading cause of cancer-related deaths, both in women and among men. In India, Several population based cohort studies were conducted which confirm the significant burden of lung cancer in India, contributing significantly toward the cancer morbidity and mortality. In order to get rid of adverse effects from currently available treatment, scientists have focused on the development of safe anti-cancer agents from herbal sources. *Sorgamara Ilai chooranam (Simarouba glauca)* is one of the herb mentioned in Siddha text with anti-cancer potential. Hence this present study was performed to validate anti-cancer potential of *Sorgamara Ilai Chooranam* through MTT assay on A549 cell line at various concentrations. Result showed that the exposure of A549 cells to *Sorgamara Ilai Chooranam* at the various concentrations significantly reduced the cell viability in a concentration dependent manner. As the concentration increases the cell viability% significantly decreases. For *Sorgamara Ilai Chooranam* from 7.8µg/ml to 62.5 µg/ml concentration the cell viability % significantly decreased from 74.85 to 52.80. In conclusion, the present study shows that *Sorgamara Ilai Chooranam* obtained from *Simarouba glauca* exhibited cytotoxic and anti proliferative activities. The anticancer activities could be explained partly by the presence of high content of Quassinoids and phenolic compounds. However, pharmacological and toxicological studies, preclinical and clinical trials are needed to elucidate the utility of *Sorgamara Ilai Chooranam* in the treatment of lung cancer.

Keywords: *Sorgamara Ilai Chooranam, Simarouba glauca, Lung cancer, A549 cell line, MTT assay.*

*Corresponding Author Email giftillda@gmail.com

Received 24 November 2018, Accepted 06 December 2018

Please cite this article as: Elsee T *et al.*, In Vitro Evaluation of Anticancer Activity of Sorgamara Ilai Chooranam (Leaves of Simarouba Glauca) Against A549 Cell Line (Human Lung Cancer Cell). American Journal of PharmTech Research 2018.

INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths, both in women and among men. Tobacco use is responsible for nearly 90 percent of all lung cancer cases. Lung cancer affects approximately 7 million people each year globally in which more than 6 million deaths result from direct tobacco use and more than 890,000 deaths result from exposure to second-hand smoke ^(1,2). The incidence and mortality of lung cancer have significantly increased worldwide ⁽³⁾. It is the leading cause of cancer death in developed countries and is rising in alarming rates in developing countries ⁽⁴⁾.

In India, Several population based cohort studies were conducted which confirm the significant burden of lung cancer in India, contributing significantly toward the cancer morbidity and mortality ⁽⁵⁾. The overall estimated lung cancer mortality in India in 2012 was 63,759, making it the third most common cause of cancer-related mortality in India after breast and cervical cancer ⁽⁶⁾.

Even though lung cancer can be treated with chemotherapy and radiotherapy, these treatments can have a negative effect on normal cells ⁽⁷⁾. In order to get rid of these adverse effects, scientists have focused on the development of safe anti-cancer agents from herbal sources. Several researches have been done so far on herbs to extract anti-cancer agents with seemingly impressive results. For many years medicinal plants have been used in Siddha system of medicine for treating various diseases, including cancer. Many single, poly-herbal formulation are available in this system of medicine to treat cancer more effectively. These drugs kill cancer cells, support defense system and detoxify the carcinogens.

Among them *Sorgamara ilai chooranam* (*Simarouba glauca*) is one of the herb mentioned in Siddha text with anti-cancer potential ⁽⁸⁾. It is also known as 'Laxmitaru' or 'paradise tree' has a long history of herbal medicine in many countries and belongs to the family *Simaroubaceae*. Because of its anti-cancer potential, it is known as tree of solace of cancer ⁽⁹⁾. The main distribution hot spots are located at tropical areas of America, Africa, Madagascar and Australia, Cuba, Brazil, Mexico, Peru, India ⁽¹⁰⁾.

Hence this present study was performed to validate anti-cancer potential of *Sorgamara Ilai Chooranam* through MTT assay on A549 cell line (Human lung cancer cell).

MATERIALS AND METHOD

Source of Collection

S. glauca leaves were collected from distinct region of Kolli hills.

Identification and Authentication of the drug

After collection the leaves were identified and authenticated by the *Gunapadam* experts in Government Siddha Medical College, Arumbakkam, Chennai – 106. The specimen sample of the herb has been preserved in PG *Gunapadam* department for future reference.

Preparation of the trial drug – *Sorgamara Ilai Chooranam*

Procedure

Fresh leaves were collected and dried at room temperature. The dried leaves were powdered by means of grinder and the powder was sieved by a cotton cloth and then bottled up. It was labeled as *Sorgamara Ilai Chooranam* (SMC).

In vitro evaluation of anticancer activity

Cell line and culture

A549 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO₂ at 37 °C.

Reagents

MEM was purchased from Hi Media Laboratories, Fetal Bovine Serum (FBS) was purchased from Cistron laboratories. Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

In Vitro assay for anti-cancer activity: (MTT assay) ⁽¹¹⁾

Cells (1 × 10⁵/well) were plated in 24-well plates and incubated in 37°C with 5% CO₂ condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/ well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl—tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 540nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC₅₀) was determined graphically. The % cell viability was calculated using the following formula:

$$\% \text{ Cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} \times 100$$

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

RESULTS AND DISCUSSION

Since the dawn of civilization, humans have been using plants, minerals and animal products as a medicine to fight against their diseases including cancer. Still, Siddha system of medicines treats many patients when other systems of medicine are no longer valid or too expensive. It should be noted that, active ingredients derived from plants always play a key role in the progress of many anticancer agents in modern medicine. Cancer for several decades has become a dominion reserved for modern medicine, thus depriving itself of all that can be traditional treatments especially herbal therapy.

In spite of recent advance in modern medicine in connection with anticancer treatments, some types of cancer remain incurable and are generally resistant to currently available treatments. In addition to that, cytotoxic agents lack specificity with respect to healthy cells in the body and can cause severe side effects. So, the search of safe, increase the specificity and effective anticancer agents still continues.

In order to contribute to the identification of new anticancer drug from Siddha system of medicine, *Sorgamara Ilai Chooranam* was selected based on traditional use previously reported in the Siddha literature. The evaluation of the cytotoxic effect of *Sorgamara Ilai Chooranam* was performed in vitro at various concentrations with respect to the A549 cell line (Human lung cancer cell) by measuring the cell viability using the MTT assay.

The percentage of cells viability was determined by calculating the optical density of treated against the control. The absorbance is read in a spectrophotometer at a wavelength of 540 nm. Comparison values are made on a basis of 50% inhibition of growth (IC₅₀) in treated cells with specific agents. Results were tabulated in Table.1 and graphically represented in Fig. 1.

Table 1: Anticancer effect of SMC on A549 cell line at various concentrations

| S. No | Concentration (µg/ml) | Dilutions | Absorbance (O.D) | Cell viability (%) |
|-------|-----------------------|-----------|------------------|--------------------|
| 1 | 1000 | Neat | 0.194 | 22.17 |
| 2 | 500 | 1:1 | 0.271 | 30.97 |
| 3 | 250 | 1:2 | 0.342 | 39.08 |
| 4 | 125 | 1:4 | 0.403 | 46.05 |
| 5 | 62.5 | 1:8 | 0.462 | 52.80 |
| 6 | 31.2 | 1:16 | 0.534 | 60.03 |
| 7 | 15.6 | 1:32 | 0.589 | 67.31 |
| 8 | 7.8 | 1:64 | 0.655 | 74.85 |
| 9 | Cell control | - | 0.875 | 100 |

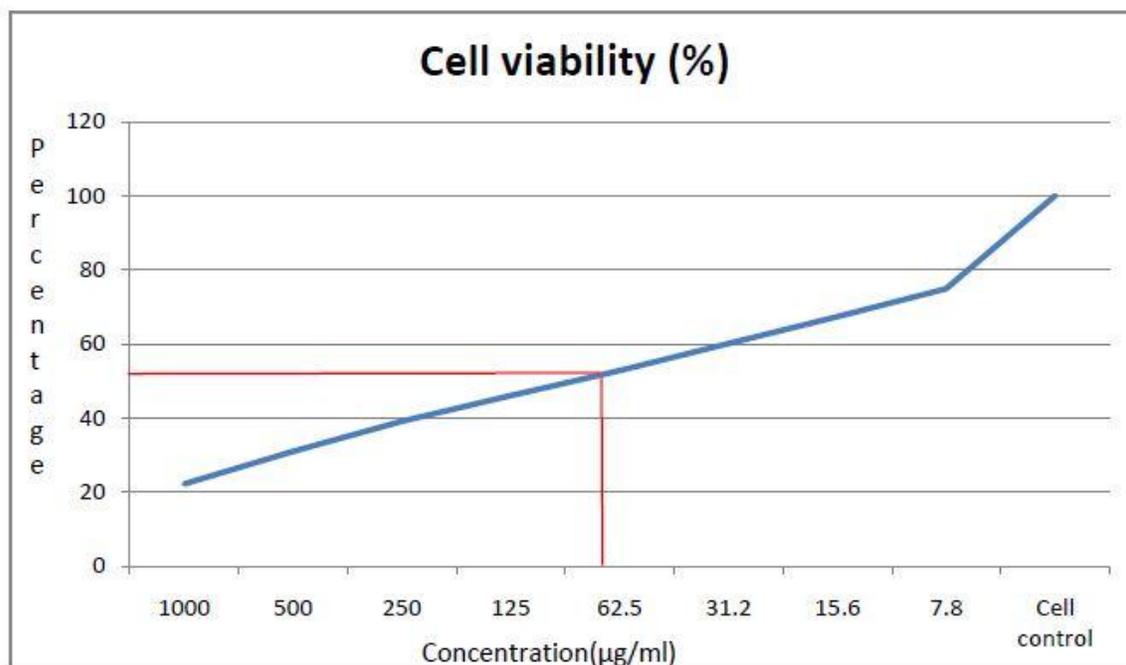
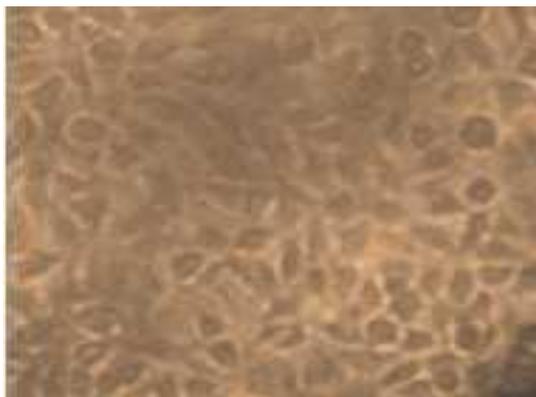


Figure 1: Percentage of cell viability of SMC at different concentration

Result showed that the exposure of A549 cells to *Sorgamara Ilai Chooranam* at the various concentrations significantly reduced the cell viability in a concentration dependent manner. As the concentration increases the cell viability% significantly decreases. For *Sorgamara Ilai Chooranam* from 7.8µg/ml to 62.5 µg/ml concentration the cell viability % significantly decreased from 74.85 to 52.80.

Some chemical constituents present in *Sorgamara Ilai Chooranam* explain these results. It contains more than 300 active substances. The components that have been identified and extracted from *Simarouba glauca* include flavonoids, glycosides phenolic compounds, saponins and fixed oil ⁽¹²⁾. *Simarouba glauca* also has 11 medicinally important quassinoids, the active principles in the tree ⁽¹³⁾.

Quassinoids are known as the bitter principles of *Simaroubaceae* plants ^(14,15, 16). Several quassinoid from *Simarouba glauca* seed have exhibited cytotoxic activity *in vitro* against KB cells (human oral epidermoid carcinoma) including glaucarubin, glaucarubinone, glaucarubol and glaucarubolone ^(17,18). Recent study has found that *Sorgamara ilai chooranam* (*Simarouba glauca*) could be useful in the treatment of cervical cancer ⁽¹⁹⁾.

**Normal A549 Cell line****Toxicity – 1000µg/m****Toxicity – 62.5µg/ml****Figure 2: Anticancer effect of Sample (SMC) on A549 Cell line**

In conclusion, the present study shows that *Sorgamara Ilai Chooranam* obtained from *Simarouba glauca* exhibited cytotoxic and anti proliferative activities. The anticancer activities could be explained partly by the presence of high content of Quassinoids and phenolic compounds.

CONCLUSION

The evaluation of the cytotoxicity of *Sorgamara Ilai Chooranam* selected in vitro, with respect to the A549 cell line (Human lung cancer cell) by measuring the cell viability using the MTT assay. The A549 cells were treated by the *Sorgamara Ilai Chooranam* at increasing concentrations for 48h. Curves representing the viability percentages as a function of the concentrations of the *Chooranam* are presented. Inhibitory concentrations at 50% (IC50) were calculated by extrapolation and the results are expressed by mean \pm SD. The curves show that there is a good dose-response correlation of the extracts for the range of concentrations tested and the cytotoxic activity is proportional to the concentration of the plant extracts studied. These data suggest that *Sorgamara Ilai Chooranam* present a strong potential for the development of anticancer agents against lung cancer.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to the Principal and Staffs of Government Siddha Medical

College, Arumbakkam, Chennai for their valuable suggestions to carry out this study successfully.

REFERENCE

1. World Health Organization. "Tobacco". Available at: [www.who.int/ media centre/ factsheets/ fs339/ en/](http://www.who.int/media/centre/factsheets/fs339/en/). Date last updated: May 2017.
2. International Association for the Study of Lung Cancer. "IASLC 2015 Statement on Tobacco Control and Smoking Cessation." September 7, 2015.
3. D. M. Parkin, F. Bray, J. Ferlay, and P. Pisani, "Global cancer statistics, 2002," *Ca—A Cancer Journal for Clinicians*, vol.55, no.2, 2005: 74–108.
4. Khuri Fr, Herbst Rs, Fossells FV. Emerging therapies in non-small-cell lung cancer. *Ann. Oncol* 2001; 12:739-44.
5. Viswanathan R, Sen Gupta, Iyer PV. Incidence of primary lung cancer in India. *Thorax*. 1962; 17:73–6.
6. Vanita Noronha et al. Lung cancer in the Indian subcontinent; *South Asian J Cancer*. 2016 Jul-Sep; 5 (3): 95–103.
7. Enger, Eldon; et al. *Concepts in Biology'* 2007 Ed.2007 Edition. McGraw-Hill. p. 173. ISBN 978-0-07-126042-8. Retrieved 23 November 2012.
8. Loganathan N. Prambariya Mooligai Marutthuva Nalavazhvu Kalangiyam, Poorvigam Arakattalai, Pondicherry -605009, 1st Edition, 2015; 103.
9. Indian Express 'A tree of solace for cancer patients' published in 'The New Indian Express' on 18th January, 2013.
10. ICRAF Agroforestry Tree Database [http:// eco crop. fao.org/ eco crop /srv /en/crop](http://eco.crop.fao.org/eco/crop/srv/en/crop). View id=9785. 2007.
11. Mosmann T (1983), Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J. Immunol. Methods* 65: 55-63
12. Sharanya VK et al. A pharmacological review on *Simarouba glauca* DC, *International Journal of Pharma Research & Review*, June 2016;5(6):32-36
13. Patil MS, Gaikwad DK, A Critical Review on Medicinally Important Oil Yielding Plant *Laxmitaru (Simarouba glauca* DC.). India. *J Pharm Sci & Res.*, 2011; 3(4): 1195-1213.
14. Guo Z, Vangapandu S, Sindelar RW, Walker LA, Sindelar RD. *Curr. Med. Chem.* 2005; 12:173–190.
15. Okano M, Fukamiya N, Lee KH. Bioactive Quassinoids. In: Atta-ur-Rahman, editor. *Studies in Natural Products Chemistry*. vol. 23. New York: Elsevier Science; 2000. pp. 285–333.

16. Murakami C, Fukamiya N, Tamura S, Okano M, Bastow KF, Tokuda H, Mukainaka T, Nishio H, Lee KH. *Bioorg. Med. Chem.* 2004; 12:4963–4968.
17. Polonsky, J., Varon, Z., Jacquemin, H., Pettit, G.R., *Experientia*, 1978, 34: 1122–1123.
18. Kupchan, S. M., Labadie, A. J., Howie, G. A., Sickles, B. R., *J Med Chem*, 1976, 19: 1130–1133.
19. Giftillda et al. Anti-cancer activiy of *Sorgamara Ilai Chooranam* (Leaves of *Simarouba glauca*) In In-Vitro Cell Line Models against Invasive Cervical Carcinoma. *World Journal of Pharmaceutical Research*. Volume 7, Issue 11, 950-957.

AJPTR is

- Peer-reviewed
- bimonthly
- Rapid publication

Submit your manuscript at: editor@ajptr.com

