IAMJ

INTERNATIONAL
AYURVEDIC
MEDICAL JOURNAL

Research Article ISSN: 2320-5091 Impact Factor: 6.719

CYTOTOXICITY STUDY OF AQUEOUS EXTRACT OF *SIMAROUBA GLAUCA* DC. LEAVES

Mahesh C D[1], Subramanya Padyanna[2], Manjunatha P M[3], Suresh Janadri[3]

[1]PhD Scholar, Sri Sri College of Ayurvedic Science and Research, Bangalore, Karnataka, India
[2]Professor, HOD, Dept of Dravyaguna, Alvas College of Ayurvedic Science and Research, Moodabidri, Karnataka, India
[3]Professor & HOD, Dept of Pharmacology, Acharya & BM Reddy College of Pharmacy, Bangalore, Karnataka, India
[3]Asso. Professor, Dept of Pharmacology, Acharya & BM Reddy College of Pharmacy, Bangalore, Karnataka, India

Corresponding Author: drmaheshcd@yahoo.co.in

https://doi.org/10.46607/iamj0110012022

(Published Online: January 2022)

Article Received: 06/12//2021 - **Peer Reviewed:** 17/12/2021 - **Accepted for Publication** 18/12/2021

ABSTRACT

Simarouba glauca DC. belongs to the family Simaroubaceae. It is a potent source of secondary metabolites. This study aimed to evaluate the cytotoxic properties of aqueous leaf extracts of *Simarouba glauca* DC. against different cell lines. Cytotoxicity of *Simarouba glauca* DC. was assessed in the aqueous leaf extract against different cell lines using MTT assay. Cell cultures are carried out with different cancer cell lines such as HL-60, HELA and MCF-7. The cancer cell lines are maintained in the logarithmic phase of growth with RPMI-1640 medium, supplemented with heat-inactivated 10% fetal bovine serum (FBS) and 1 % penicillin incubated in 5 % CO2 incubator and 95 % humidified air. MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] is a quantitative standard colorimetric assay used to measure mammalian cellular growth, cell survival and cell proliferation based on the ability of cells alive. This assay determines the cytotoxicity of potential medicinal agents and other toxic materials. *Simarouba glauca* DC. leaves extract shows *in vitro* anticancer activity at 285 µg/ml (HL-60),

260 µg/ml (HELA) and 280 µg/ml (MCF-7) concentrations. In terms of efficacy compared to standard 5-FU is less anti-cancer activity.

Keywords: *Simarouba glauca*, cytotoxic, anticancer activity

INTRODUCTION

Cancer is a dreadful disease and a major public health problem in the world. Plant-derived drugs are used as one of the treatments for cancers. Plants have been used for medicinal purposes since the ancient period. The use of medicinal plants in cancer prevention and management is common all over the world passed down from generation to generation.[1] Traditional medicines are widely practised. Being cost-effective and inducing lesser side effects are the benefits that led to the use of plant materials as a source of medicines for various human ailments.[2] *Simarouba glauca* DC. is an evergreen edible oil tree belonging to the family Simaroubaceae. This family includes 32 genera and more than 170 species of trees and bushes of pantropical distribution.[3] Its common names are Lakshmi Taru and paradise-tree. A molecular phylogeny of the family was reported in 2007, data revealed the relationships within the family.[4] The Simaroubaceae family possess a wide variety of chemicals and can be characterized as having potential bioactive molecules with remarkable research potential. To substantiate the potentiality and reported in 1961. The first quassinoide structure was elucidated. More than 200 currently known chemicals are isolated and identified from Simaroubaceae family.[5] Most of these secondary metabolites showed potential biological activities in bioassay systems posing structures that could be considered as effective therapeutic and bioactive agents.[6] The cultivation of *Simarouba Glauca* DC. is in semi-arid dry and saline land areas of Indian states, such as Gujarat, Tamilnadu, Maharashtra, Karnataka, and Andhra Pradesh. *Simarouba glauca* DC. tree usually grows in marginal wastelands, drylands, and degraded soil. This is an evergreen large fruit tree where birds perch and deposit the seeds near subtropical moist forest plants. This tree is a medium-sized evergreen tree that begins to bear fruit when it is 6-8 years old and continues until 4-5 years later. Flowering takes place from December to February. This tree is polygamodioecious, and most of the female ones are good bearers. Bitter content substances in this tree have pharmaceutical properties (Fernando and Quinn, 1992; Muhammad et al., 2004.[7] The principal geographical distribution of this tree is in tropical America, extending to West Africa, Madagascar, Asia (Malaysia), and some Pacific regions.[8] This family is represented by the genera Quassia and Picrolemma in Brazil. Castela and Picrasma in Amazon, to the South and Simaba, Simarouba and Picrolema, which are present throughout the country.[9] *Simarouba glauca* DC. is native to Southern Florida, the West Indies, and Brazil (Cronquist, 1944). It grows under tropical conditions in Central America spreading from Mexico to Panama Southern Florida and Caribbean Islands. *Simarouba glauca* DC. was introduced in Kenya and Burundi in Africa in 1957.[10] The cultivation of *Simarouba glauca* DC. extended to semiarid dry and saline land areas of other Indian states like Gujarat, Tamil Nadu, Maharashtra, Karnataka and Andhra Pradesh. *Simarouba glauca* DC. tree can grow well even in marginal wastelands and dry land with degraded soil.[11] This study highlighted the significance of the anti-cancer and cytotoxic effect of aqueous extracts of *Simarouba glauca* DC. on cancer cell lines.

Materials and Methods:

Collection of Plant Material: Fresh leaves of *Simarouba glauca* DC. were collected from the herbal garden of Sri Sri College of Ayurvedic Science and Research, Kanakapura Road, Bangalore. The leaves were washed thoroughly 2 to 3 times with water and with autoclaved distilled water and chopped into small pieces. The cut leaves were divided into two lots: Fresh leaves of *Simarouba glauca* DC. (FL) and dried leaves of *Simarouba glauca* DC. (DL).

Identification and Authentication of the drug: The genuinity of the plants (stem part) was confirmed by Dr Shivamanjunath, Botanist Department of Dravyaguna, Sri Sri College of Ayurvedic Science and Research. The specimen sample of the herb has been preserved in Dravyaguna PG Department for future reference.

Solvent Extraction: Thoroughly washed dried leaves and fresh leaves of *Simarouba glauca* DC. were powdered with the help of blender, 5 g dried leaf powder was mixed in 100 ml of distilled water. The extraction was successfully done by Soxhlet extractor for 48 hrs. The solvent extracts were concentrated and reduced by a rotary vacuum evaporator and preserved in airtight bottles at 5° C until further use.[12]

MTT Assay:

Cell cultures are carried out with different cancer cell lines such as HL-60, HELA and MCF-7. The cancer cell line is maintained in the logarithmic phase of growth with RPMI-1640 medium, supplemented with heat-inactivated 10% fetal bovine serum (FBS) and 1 % penicillin incubated in 5 % CO2 incubator and 95 % humidified air. MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] is the quantitative standard colorimetric assay used to measure mammalian cellular growth, cell survival and cell proliferation based on the ability of cells a life. This assay determines the cytotoxicity of potential medicinal agents and other toxic materials. The pale yellow of MTT enters inside the mitochondria of the viable cell and get reduced enzymatically to form dark colour formazan crystal by cleaving tetrazolium rings. The formazan crystal is insoluble in an aqueous solution. Therefore, it is treated with an organic solvent like acid-isopropanol (0.04 N Hydrochloric in isopropanol) to dissolve and produce a purple formazan product (colour solution) and the absorbance was taken from 490 nm to 600 nm by using the ELISA reader.

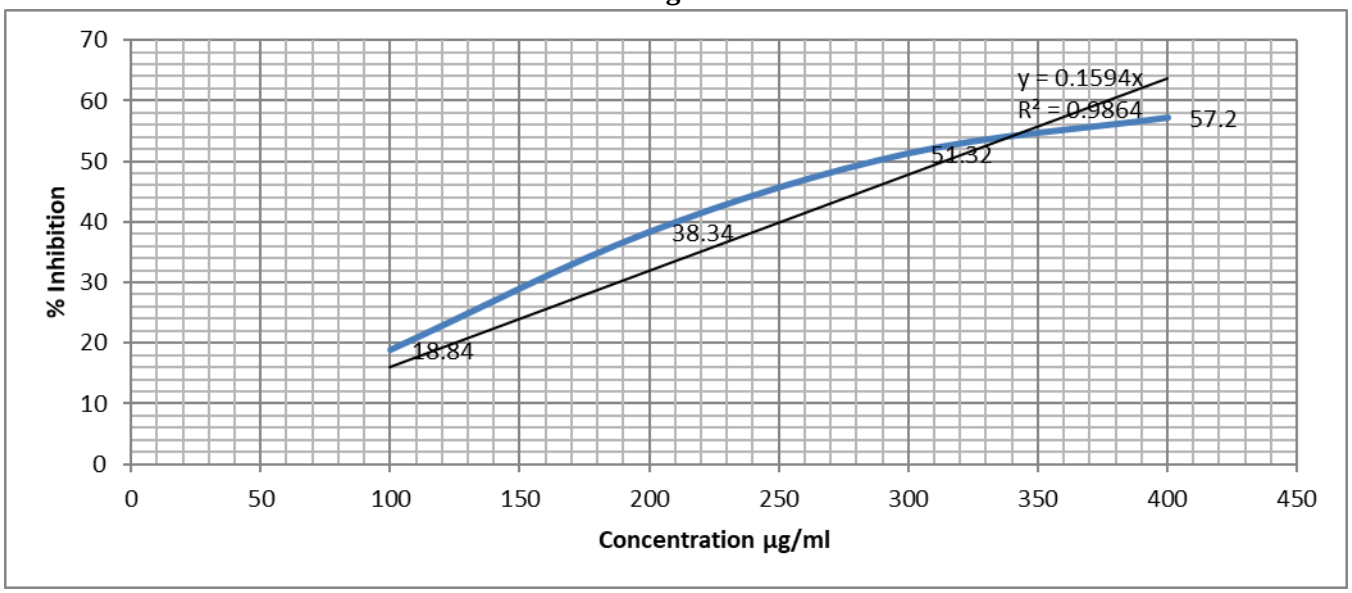
***in vitro* model on leukaemia (HL-60) cell line:[13]**

Table 1: Effect of different concentrations of *Simarouba glauca* DC. leaves extract and standard 5 FU drug on leukaemia (HL-60) cell line

Drugs	**% Inhibition at 100µg**	**% Inhibition at 200 µg**	**% Inhibition at 300µg**	**% Inhibition at 400 µg**	**IC50 µMol**
Simarouba glauca DC. leaves extract	18.84 ± 0.27	38.34 ± 0.28	51.32 ± 0.66	57.20 ± 0.40	**285**

All the values were expressed in Mean ± SEM (n=3).

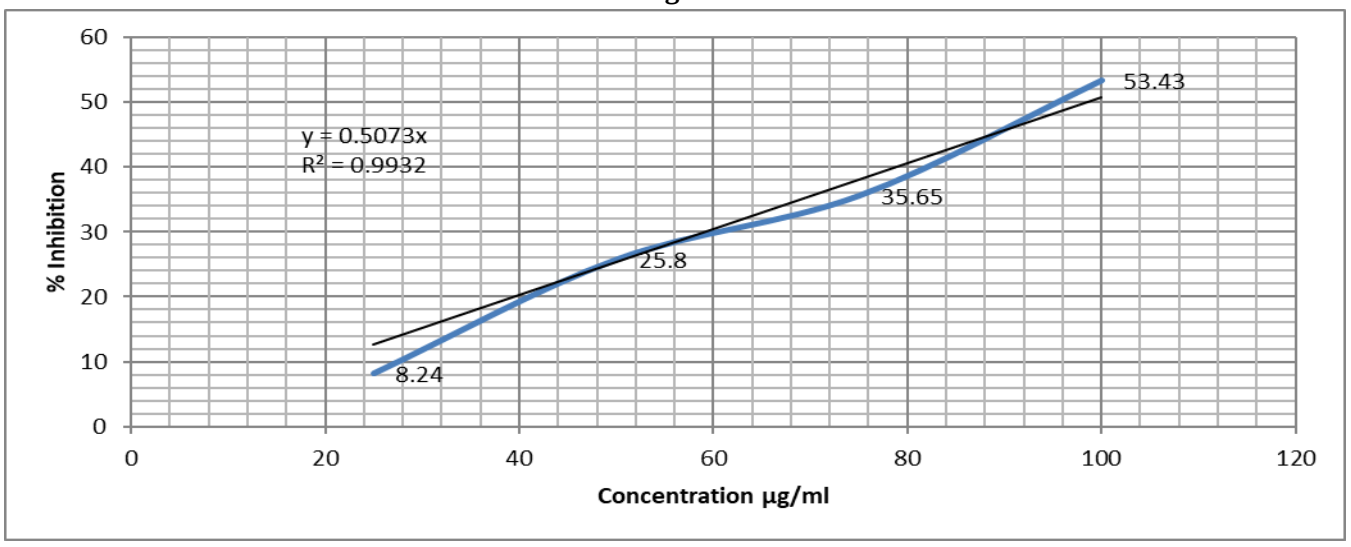
Fig: 1



Drugs	% Inhibition at 25 µg	% Inhibition at 50 µg	% Inhibition at 75 µg	% Inhibition at 100 µg	IC50 µMol
5 FU	8.24 ± 0.64	25.8 ± 0.45	35.65 ± 0.21	53.43 ± 0.24***	**96**

All the values were expressed in Mean ± SEM (n=3).

Fig: 2



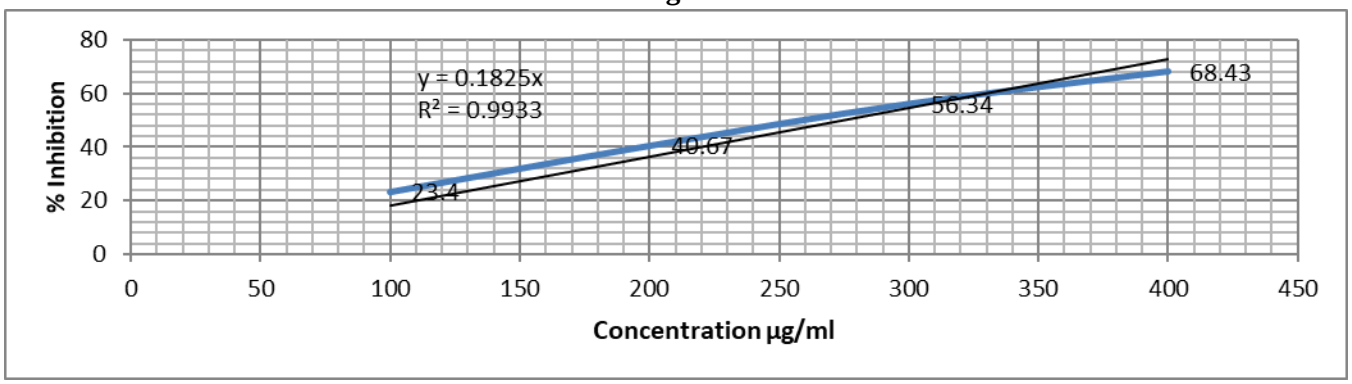
The *in vitro* model cervical cancer (HELA) cell line:[14]

Table 2: Effect of different concentrations of *Simarouba glauca* DC. leaves extract and standard 5 FU drug on cervical cancer (HELA)

Drugs	% Inhibition at 100 µg	% Inhibition at 200 µg	% Inhibition at 300 µg	% Inhibition at 400 µg	IC50 µMol
Simarouba glauca DC. leaves extract	23.4 ± 0.36	40.67 ± 0.16	56.34 ± 0.23	68.43 ± 0.67	**260**

All the values were expressed in Mean ± SEM (n=3).

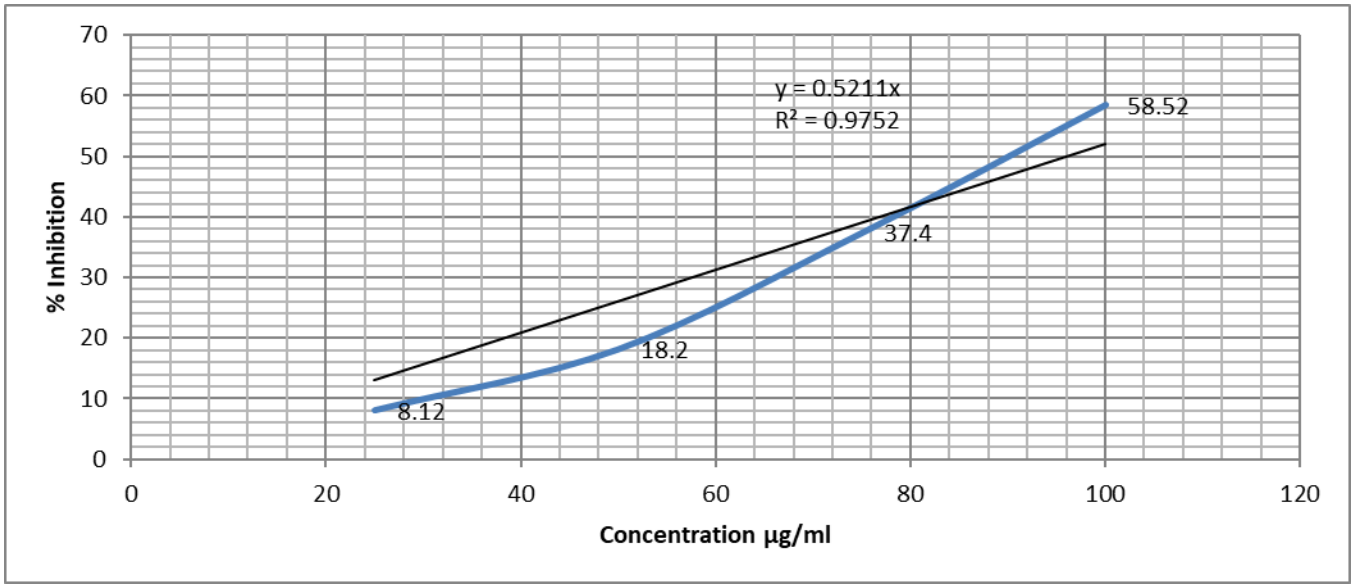
Fig 3:



Drugs	% Inhibition at 25 µg	% Inhibition at 50 µg	% Inhibition at 75 µg	% Inhibition at 100 µg	IC50 µMol
5 FU	8.12 ± 0.15	18.2 ± 0.12	37.4 ± 0.23	58.52 ± 0.30***	**90**

All the values were expressed in Mean ± SEM (n=3).

Fig 4:



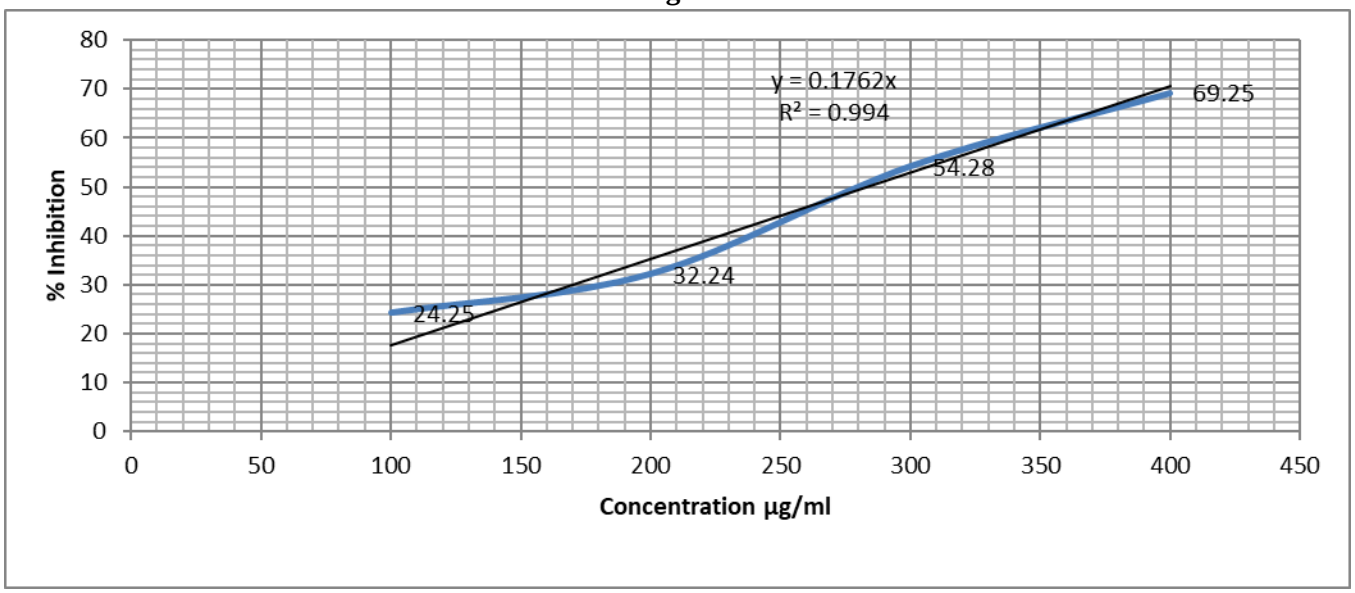
The *in vitro* model Breast cancer (MCF-7) cell line:[15]

Table 3: Effect of different concentrations of *Simarouba glauca* DC. leaves extract and standard 5 FU drug on Breast cancer (MCF-7)

Drugs	% Inhibition at 100 µg	% Inhibition at 200 µg	% Inhibition at 300 µg	% Inhibition at 400 µg	IC50 µMol
Simarouba glauca DC. leaves extract	24.25 ± 0.55	32.24 ± 0.22	54.28 ± 0.15	69.25 ± 0.25	**280**

All the values were expressed in Mean ± SEM (n=3).

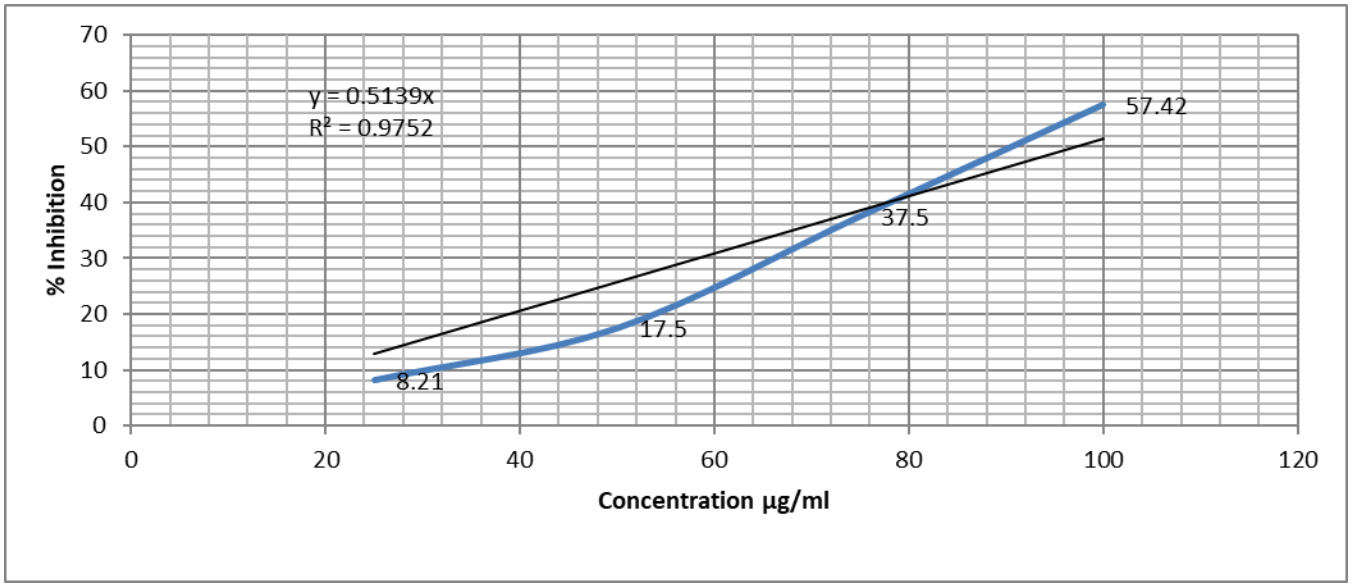
Fig 5:



Drugs	% Inhibition at 25 µg	% Inhibition at 50 µg	% Inhibition at 75 µg	% Inhibition at 100 µg	IC50 µMol
Imantinib Mesylate	8.21 ± 0.10	17.5 ± 0.20	37.5 ± 0.62	55.42 ± 0.26***	**92**

All the values were expressed in Mean ± SEM (n=3).

Fig 6:



DISCUSSION

Medicinal plants are important resources of phytochemicals and herbal drugs. Pharmaceutical companies have screened more than 25,000 plants for developing anti-cancer drugs. The herbal system of medicine has been applied for thousands of years.[16] Derivatives of natural products represent approximately 60% of all chemotherapeutic agents approved by the Food and Drug Administration (FDA); for example, vincristine, vinblastine, and Taxol.[17] Quas-

sinoides are a group of compounds extracted from the plants of the Simarubaceae family, which have been seen used for many years in folk medicine. The molecules gained no toxicity after the initial discovery of the anti-leukemic activity of one membrane, Bruceantin in 1975, currently, over 150 quassinoids have been isolated and classified based on their chemical structures and biological properties investigated *invitro* and *invivo*. Many molecules display a wide range of inhibitory effects, including anti-inflammatory, antiviral, anti-malarial and anti-proliferate effects in various tumour cell types. SG contains compounds with properties that suppress tumours. The herb's antileukemic and antitumor role has been linked to four Quassinoids namely Ailanthinone, Glaucarubinone, Dehydroglaucarubinone, and Holacanthone.[18] The extracts were screened for cytotoxicity using an MTT assay. Maximum cytotoxicity was observed at the highest concentration of 400 µg/ml in SG extract on the different cell lines. The findings of this study indicated that MTT assay offered the convenience of providing drug sensitivity information. Given that appropriate cytotoxicity was proved for cancer cell lines treated with HL-60, HELA and MCF-7 of SG. Further research of SG extract can be confirmed by gene expression study in different cancer cell lines.

CONCLUSION

The aqueous extracts of *Simarouba glauca* DC leave the show *in vitro* anticancer activity at 285 µg/ml (HL-60), 260 µg/ml (HELA) and 280 µg/ml (MCF-7) concentrations. In terms of anticancer efficacy compared to standard 5-FU is less anti-cancer activity.

Acknowledgement

The authors are thankful to the management of Sri Sri College of Ayurvedic Science and Research, Bangalore and Acharya & BM Reddy College of Pharmacy, Bangalore.

REFERENCES

1. Bidavikas, Sujathankanjiranan, Sujasomashakeranan Nair Rajan, Sukumaran Anil (2011) The apoptosis of leaf extracts of *Simaruba glauca* against Human leukemic cancer cells, Asian pacific journal of cancer prevention vol 22 1305.
2. Almeida MMB, Arriaga ÂMC, Santos AKLd, et al (2007). Ocorrência e atividadebiológica de quassinóides da últimadécada. Química Nova, 30, 935-51.
3. Alves IABS, Miranda HM, Soares LAL, et al (2014). Simaroubaceae family: botany, chemical composition and biological activities. Revista Brasileira de Farmacognosia, 24, 481-501.
4. Clayton J W, Fernando E S, Soltis P S, et al. Molecular phylogeny of the 3 of heaven family (Simaroubaceae) based on chloroplast and nuclear marker. 2001; 168:1325-39. (Google scholar).
5. Balu KE, Ramya KS, Radha A, et al (2020). Structure of intact chitinase with hevein domain from the plant *Simarouba glauca*, known for its traditional anti-inflammatory efficacy. Int J Biol Macromol, 161, 1381-92.
6. Vikas B, Akhil BS, Suja SR, et al (2017). An exploration of phytochemicals from Simaroubaceae. Asian Pac J Cancer Prev, 18, 1765-7.
7. Muhammad I, Bedir E, Khan SI, et al (2004). A new antimalarial quassinoid from Simabaorinocensis. J Nat Prod, 67, 772-7.
8. Simão SM, Barreiros EL, Da Silva MFdGF, et al (1991). Chemogeographical evolution of quassinoids in simaroubaceae. Phytochemistry, 30, 853-65.
9. Arriaga AC, de Mesquita AC, Pouliquen YB, et al (2002). Chemical constituents of Simaroubaversicolor. An Acad Bras Cienc, 74,415-24.
10. Joshi S, Joshi S (2002). Oil tree-Laxmitaru glauca. University of Agricultural Sciences, Bangalore and Indian council of Agricultural Research, New Delhi, India.
11. Govindaraju K, Darukeshwara J, Srivastava AK (2009). Studies on protein characteristics and toxic constituents of *Simarouba glauca* oilseed meal. Food Chem Toxicol, 47, 1327-32.
12. The Ayurvedic Pharmacopoeia of India Part 2. Vol 1. 1st edition. New Delhi: Ministry of Health & Family Welfare, Dept. of AYUSH, GOI. 2007:144-6.
13. Abendanza T S, Oliveira C R, Barbosa C M V, Pereira F E G, Cunha R L O R, Caires A C F, et al. Bcl-2 expression and apoptosis induction in human HL60 leukaemic cells treated with a novel organotellurium (IV) compound RT-04. Food Chem Toxicol. 2008;46(7):2540-5. (Pubmed) (Google scholar).
14. Terry S. 'HeLa' Herself. The Scientist. 2006:20-22.

15. Levenson A S, Jordan V C. MCF-7: the first hormone – responsive breast cancer cell line. Cancer Res. 1997;57(15):3071-8. (Pubmed) (Google scholar).
16. Sakarkar D, Deshmukh V (2011). Ethnopharmacological review of traditional medicinal plants for anti-cancer activity. Int J Pharm Tech Res, 3, 298-308.
17. Newman LA (2014). Breast cancer. Surg Oncol Clin N Am, 23, xv-xvi.
18. Jach, ME, Laureysens I, Ceulemans, R. (2000) Above and below-ground production of young scots pine (Pinus sylvestris L.) Trees after three years of growth in the field under elevated CO2. Annals of Botany. 85(6), 789–798.

Source of Support: Nil
Conflict of Interest: None Declared

How to cite this URL: Mahesh C D et al: Cytotoxicity Study Of Aqueous Extract Of Simarouba Glauca Dc. Leaves. International Ayurvedic Medical Journal {online} 2021 {cited January 2022} Available from: http://www.iamj.in/posts/images/upload/01_08.pdf