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Invitro Cytotoxic Studies of crude methanolic extract of *Saraca indica* bark extract

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Abstract: *Saraca asoca Roxb. De Wilde – Ashoka is a Sanskrit word which means" without sorrow" or that gives no grief. Ashoka tree, universally known by its binomial Latin name Saraca asoca (Roxb.),De wild or Saraca indica belonging family Caesalpinacea. It is a small evergreen tree 7-10cm high. It occurs up to the altitude 750m. The plant is found to have spasmogenic, oxytocic, uterotonic, antibacterial, anti-implantation, antitumor, antiprogestational, antioestrogenic activity. The objective of this study is to evaluate the invitro cytotoxicity of the crude methanolic bark extracts of Saraca indica on HeLa cell lines using MTT assay. The extract possessed highly significant percentage of inhibition on the cell lines and the IC50 value was determined to be 14.63µg/ml. These results show a significant antitumor and cytotoxic effect of extract against human cervical cancer HeLa cell line and support the ethnomedical use of Saraca indica.*
Keywords: *HeLa, IC50, Invitro,MTT*

I. Introduction

Humankind first utilized natural materials found in environment on an empirical basis to cure various ailments. Allopathic medicine may cure a vast range of diseases; however, its high prices and side-effects are causing many people to return alternative medicines which have fewer side effects. The interest in medicinal plants and their biologically active derivatives has been tremendously increased; in relation to the possible development of novel potential drugs for several pathologies of social impact [1, 2].Plants have been a source of medicinal substances for thousands of years. Plants and phytoproducts continue to play a vital role in the treatment of various diseases including cancer. Many natural substances of plant origin are reported to be biologically active, endowed with antimicrobial and antioxidant properties [3].The antitumor activity of medicinal plants and its possible application in cancer prevention has been recently reported[4,5].Several studies have established a link between phytochemicals and the range of biological activities that impart health benefits in human beings. These phytochemicals present in plants are produced as primary and secondary metabolites. Drug discovery from plants is a multi-disciplinary approach which combines various botanical, ethno-botanicals, phytochemicals and biological and chemical separation techniques. [6] However, despite these observations, it is significant that over 60% of currently used anti-cancer agents are derived from natural sources, including plants, marine organisms and micro-organisms [7, 8].

In 2010, WHO Globocan cancer registry accounted 12.7×10^6 new cases and 7.6×10^6 fatalities per year due to cancer, making cancer as the second leading cause of mortality in high-income countries. In contrast to treatment of cardiovascular diseases, for which modern pharmacology brought tremendous improvements, the treatment options for cancer (chemotherapy, hormone therapy drugs, radiation therapy or their combinations) have barely improved the death rate from cancer, despite years of research [9].Cervical cancer remains the third most common cancer in women worldwide [10] and the leading malignancy in developing countries, accounting for 83% of whole cancer cases. Although well-organized screening and early therapeutic schedule have been carried out, the occurrence of invasive cervical cancer is still common, especially in developing areas. It is well known that persistent infection of High-Risk Human Papilloma Virus (HR-HPV) is a necessary causal event in cervical carcinogenesis [11].

Human cancer cell lines have been the most commonly used experimental models because they retain characteristic features of cancer cells, purity, are easily propagated and can be genetically manipulated to provide reproducible results; results obtained with cell lines are often extrapolated to human tumors *in vivo*. In past years, a number of methods have been developed to study cell viability and proliferation in cell culture. [12].Cytotoxicity assays have been developed which use different parameters associated with cell death and proliferation [13]. Tetrazolium salts [14] are reduced only by metabolically active cells. Thus, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) can be reduced to a blue colored formazan[15].The amount of formazan produced is directly proportional to the number of active cells in the culture, MTT and other tetrazolium salt have been used in cellular proliferation and cytotoxicity assays [16].

Saraca asoca Roxb. de Wilde, syn. *S. indica* auct non L. (Ashoka) is an evergreen tree belonging to the Caesalpiniaceae subfamily of the legume family [17].Several studies has reported that the plant have uterotonic,

antibacterial,antitumor and antioestrogenic activity [18].In this study the bark of the plant has been studied for *invitro* anticancer activity.

II. Materials & Methods

The study was carried out in the PG and Research Laboratory, Department of Microbiology of SreeNarayana Guru College of Arts and Science College, Coimbatore and Cell culture laboratory of KMCH College of Pharmacy, Coimbatore.

2.1. Collection of sample

The bark of the *Saraca indica* tree was collected and washed with water to remove the adhering impurities.

2.2. Collection of cell line

The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune.

2.3. Sample Processing

The washed bark sample has been cut into small pieces and then allowed to shade dry until the water content has been lost. The dried samples were then pulverized using electric blender and the finely powdered samples were stored in air tight containers. . 200 g of the powdered samples were extracted with methanol by Soxhelt apparatus. The extracts were completely dried and used for the study.

2.4. *Invitro* cytotoxicity

Invitro cytotoxic activity was determined using standard MTT assay. The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity.

2.4.1. Cell Treatment Procedure

The monolayer cells were detached with trypsin-ethylenediamine tetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microliters per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 hours, the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in neat dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microliters per well of each concentration was added to plates to obtain final concentrations of 50, 25, 12.5, 6.25 and 3.125 µg/ml. The final volume in each well was 200 µl and the plates were incubated at 37^0C, 5% CO_2, 95% air and 100% relative humidity for 48h. The medium containing without samples were treated with DMSO served as control. Triplicate was maintained for all concentrations.

2.4.2. MTT Assay

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells.After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

In terms of cytotoxicity, lower the IC 50 higher the cytotoxicity [19].

III. Results

HeLa cervical cancer cells were seeded and exposed to *Saraca indica* methanol extracts at 50, 25, 12.5, 6.25 and 3.125µg/ml of concentration.*Saraca indica* methanol bark extracts inhibited cell growth in HeLa in dose-dependent manners(Fig 1).The extracts exposure demonstrated a maximum decrease in cell growth of HeLa cancer cell lines of 90.71% of inhibition at 50ug/ml concentration of the extract compared to DMSO control treatment(Table1).In HeLa cells, the methanolic bark extracts demonstrated a reduction in proliferation of cancer cells as the concentration increased after two days of exposure. As shown in Fig 2, a readily observed dose-response in cell growth inhibition was seen in HeLa ervical cell lines as *Saraca indica* methanol concentration increased. The IC50 value has been determined as 14.63µg/ml using Graph Pad Prism software.

IV. Discussion

Cancer of the cervix is the third most common malignancy worldwide in women, and the most common gynecologic cancer in the developing world. Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity.*Saraca indica* is an important plant of Indian system of medicine for its chemical constituents and its well-known pharmacological activities. Catechin is a well-known flavonoid found in this plant and also in many foods plants and often utilized by naturopaths for the symptomatic treatment of several gastrointestinal, respiratory and vascular diseases[20].Five lignan glycosides, lyoniside, nudiposide,5-methoxy-9-β-xylopyranosyl-(-)-isolariciresinol,icarisideE3, and schizandriside, and three flavonoids, (-)-epicatechin, epiafzelechin-(4β-8)-epicatechin and procyanidine B2, together with β-sitosterol glucoside has been isolated from the bark of this plant[21]. Many studies on health benefits have been linked to the catechin content. (+)-Catechin shown to possess antibiotic properties due to their role in disrupting a specific stage of the bacterial DNA replication process [22], and possesses anti-carcinogenic effects [23]. (+)-Catechin has been shown to have antioxidant [24] and has also been reported to induce cancer preventive activity mediated through a chaperone like property [25].Polyphenol E and epigallocatechin gallate,found in green tea has been found to inhibit the growth of HPV-18 immortalized cervical cells and cervical cancer cell lines.[26,27].Similarly in this study it has been observed clearly that the bark extracts of *Saraca indica* has significant anticancerous activity against HeLa cervical cancer cell lines. This activity may be due to the presence of various flavonoids and polyphenolic components in the bark.

V. Figures And Tables

Table 1. Percentage cell inhibition of *Saraca indica* against HeLa Cervical Cancer cell line

Concentration of Plant Extract (µg/ml)	Absorbance at 572 nm	Average	Percentage Cell Inhibition
3.125 µg	0.365	0.377333	5.271967
	0.379		
	0.388		
6.25 µg	0.343	0.339667	14.72803
	0.34		
	0.336		
12.5 µg	0.214	0.212667	46.61088
	0.204		
	0.22		
25.0 µg	0.115	0.119333	70.04184
	0.114		
	0.129		
50.0 µg	0.033	0.037	90.7113
	0.038		
	0.04		
Control	0.371	0.398333	-
	0.417		
	0.407		

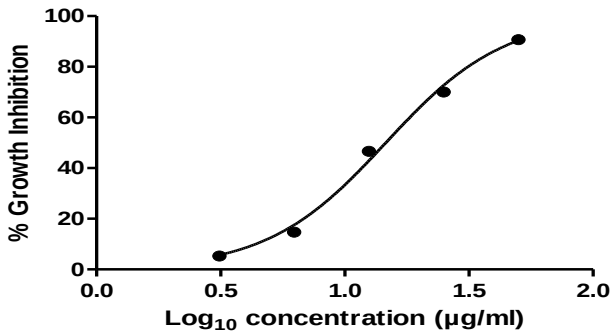

Figure 1: Percentage of growth inhibition of methanol bark extract of *Saraca indica* of various concentrations against HeLa Cervical Cancer cell lines

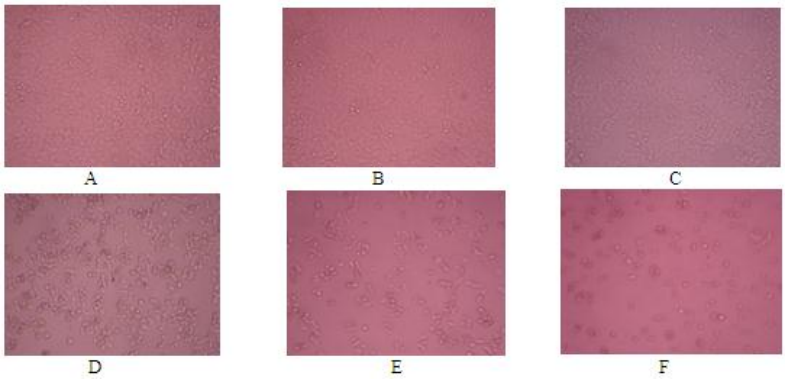

Figure 2: MTT Assay for various concentrations of methanolic extracts of *Saraca indica* bark in HeLa cell lines

A: MTT assay of normal HeLa cell lines
B: Cells treated with 3.125 µg of methanolic bark extract of *Saraca indica*
C: Cells treated with 6.25 µg of methanolic bark extract of *Saraca indica*
D: Cells treated with 12.5 µg of methanolic bark extract of *Saraca indica*
E: Cells treated with 25 µg of methanolic bark extract of *Saraca indica*
F: Cells treated with 50 µg of methanolic bark extract of *Saraca indica*

VI. Conclusion

The results obtained in this MTT assay shows that the bark of *Saraca indica* has significant cytotoxic activity against HeLa cell lines. Further investigations may lead to the development of potent anticancerous agents.

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References

[1] I Hedberg,Botanical methods in ethnopharmacology and the need for conservation of medicinal plants, Journal of Ethnopharmacology, 38, 1993,121–128.
[2] M Heinrich, S Gibbons, Ethnopharmacology in drug discovery: an analysis of its role and potential contribution. Journal of Pharmacy and Pharmacology, 53, 2001, 425–432.
[3] A Ankli , M Heinrich , P Bork , LWolfram , P Bauerfeind , R Brun , C Schmid ,C Weiss ,R Bruggisser ,J Gertsch , M Wasescha and O Sticher,Yucatec Mayan Medicinal Plants: evaluation based indigenous uses, Journal of Ethanopharmacology, 79,2002,43-52.
[4] N Katsube , K Iwashita,T Tsushida ,K Yamaki and M Kobori , Induction of apoptosis in cancer cells by Bilberry (Vaccinium myrtillus) and the anthocyanins,Journal of Agricultural and food chemistry,51,2003,68-75.
[5] HF Chiu, YC Wu, Cytotoxic constituents of the stem bark of Neolitsea acuminatissima.,Journal of Natural Products, 65,2002, 255-258
[6] S.M Jachak. and A Saklani, Challenges and opportunities in drug discovery from plants, Current science, 92(9), 2007, 1251-57 .
[7] G.M Cragg, D.G.I. Kingston and D.J Newman. (Eds.), Anticancer Agents from Natural Products Boca Raton,Florida: Taylor and Francis ,2005.
[8] Singh Shachi, Antimiotic activity of a New Compound Isolated from the Flower of Prosopis juliflora, Research Journal of recent Science 1(6), 2012,22-26 .
[9] J Ferlay , HR Shin , F Bray , et al. GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer, 2010. Available from: http://globocan.iarc.fr.
[10] A Jemal ,F Bray MM Center ,J Ferlay , E Ward , D Forman , Global cancer statistics. CA Cancer J Clin,Mar-Apr; 61(2),2011,134.
[11] DM Parkin, F Bray, J Ferlay and P Pisani, Global cancer statistics, 2002. CA Cancer J Clin, 55(2), 2005, 74-108.
[12] Cook J.A, Mitchell and J.B, Viability measurements in mammalian systems, Anal. Biochem. 179, 1989, 1–7.
[13] J¨org Weyermanna, Dirk Lochmanna, Andreas Zimmer, A practical note on the use of cytotoxicity assays,International Journal of Pharmaceutics 288,2005, 369–376.
[14] Smith, F.E,Tetrazolium Salt.Science 113, 1951, 751–754.
[15] T Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, Journal Immunol. Methods 65, 1983, 55–63.
[16] H Kim, Yoon, SC., T Y Lee, Jeong D, Discriminative cytotoxicity assessment based on various cellular damages, Toxicology Letter, 184, 2009, 13-17.
[17] V. V. Sivarajan, I. Balachandran, Ayurvedic drugs and their plant sources. New Delhi:Oxford and IBH publishing Co. Pvt. Ltd, 1994, 57.
[18] http://www.divineremedies.com.
[19] Hans Vogel, Drug Discovery and Evaluation:Pharmacological assay, 2007,Springer Publisher, 1334.
[20] Permender Rathee, Sushila Rathee, Deepti Rathee and Dharmender Rathee,Quantitative estimation of (+)-Catechin in stem bark of Saraca asoka Linn using HPTLC,Der Pharma Chemica, 2(1),2010,306-314.

[21] Samir Kumar Sadhu, Amina Khatun,Panadda Phattanawasin et al,Lignan glycosides and flavonoids from Saraca asoca with antioxidant activity, J Nat Med 61,2007,480-482
[22] H. Gradisar, P. Pristovsek, A. Plaper, R. Jerala, J. Med. Chem., **,** 50 (2),2007,264.
[23] Science Daily, 31 May 2008.
[24] Y. Dobashi, T. Hiranoa, M. Hiranoa, Y. Ohkatsua. J. Photochem. Photobiol. A: Chem., 2008**,** 197, 141.
[25] T. Kuzuharaa, M. Suganumab, H. Fujikia, Cancer Letters, 2008**,** 261, 12.
[26] WG Li, QH Li and Z Tan, Epigallocatechin gallate induces telomere fragmentation in HeLa and 293but not in MRC-5 cells, Life Sci;76(15), 2005,1735–1746.
[27] M Yokoyama, M Noguchi, Y Nakao, A Pater and T Iwasaka,The tea polyphenol, (-)-epigallocatechingallate effects on growth, apoptosis, and telomerase activity in cervical cell lines,Gynecol Oncol,92(1), 2004, 197–204.