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akilapharma.23@gmail.com***IN-VITRO* ANTI TUMOUR ACTIVITY OF *VITEX LEUCOXYLON* LINN USING MTT ASSAY METHOD****Akila Elias, Priya John and Archana chandran****ABSTRACT**

Indigenous medicines for their known least side effects than allopathic medicine have been investigated for unsolved problems in diseases like Cancer, AIDS. This study aims to evaluate the Anti-tumour activity of various extracts of leafs of *Vitex leucoxyton* by *invitro* method using MTT assay method. Plant extracts were screened for its cytotoxicity against HeLa cell lines at different concentrations to determine the IC₅₀. Ethanol & Chloroform extracts showed more significant effect on the HeLa cell line when compared to Petroleum ether extract.

Keywords: Anti-tumour activity, cytotoxicity, HeLa cell lines, MTT assay, *Vitex leucoxyton*.

INTRODUCTION

The plant *Vitex leucoxyton* Linn (Family: Verbenaceae) is widely available in TamilNadu and it is a deciduous tree grows upto 15m tall. The leaves are compound, digitate or rarely trifoliolate, minutely pubescent and leaflets 5. The leaflets are elliptic, apex acute to obtuse, base cuneate-attenuate, margin entire, chartaceous or thinly coriaceous, glaucous beneath, glabrous, midrib canaliculated above. The leaves are traditionally used for the treatment of leprosy, cancer, emetic, headache. The present study was carried out to evaluate the *in-vitro* anti tumour activity of various extracts of leaves *Vitex leucoxyton* Linn using MTT assay method [1].

MATERIALS AND METHODS**Plant material**

The leaves of plant of *Vitex leucoxyton* Linn were collected from Tirunelveli District, Tamilnadu, during July 2011. Leaves

were collected in fine dry weather and were dried in sunshade for a week. The plant was identified and authenticated by Prof. P.Jayaraman, (Reg.No.PARC/2012/1135).The shade dried plant material was coarsely powdered and used for preparation of three different extracts like petroleum ether, chloroform and ethanol extract for further studies.

Cell line used:

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°C, 5% CO₂, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

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 [2,3].

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic 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 1x10⁵

cells/ml. One hundred microlitres 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°C, 5% CO₂, 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 dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 1000, 500, 250, 125 and 62.5 µg/ml. The final volume in each well was 200 µl and the plates were incubated at 37°C, 5% CO₂, 95% air and 100% relative humidity for 48hours. The medium containing without samples were served as control. Triplicate was maintained for all concentrations. After 48hours of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measure the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula [4-7].

$$\% \text{ cell Inhibition} = 100 - \frac{\text{Abs (sample)}}{\text{Abs (control)}} \times 100.$$

Nonlinear regression graph was plotted between % Cell inhibition and Log₁₀ concentration and IC₅₀ was determined using GraphPad Prism software.

RESULTS AND DISCUSSION

The cytotoxicity study was carried out by MTT assay for plant extract of *Vitex leucoxylo*n leaves. These extracts were

screened for its cytotoxicity against HeLa cell lines at different concentrations to determine the IC₅₀ (50% growth

inhibition).Results are tabulated and Graphically represented. The percentage growth inhibition was found to be increasing with increasing concentration of test compounds and that show in Figure The IC₅₀ value of Pet. Ether, Chloroform and Ethanol extract on the HeLa cell line were found to

be 436.7, 383, 277.4 µg/ml and R² values were 0.987, 0.9952, 0.9899 respectively. Ethanol & Chloroform extracts showed more significant effect on the HeLa cell line when compared to Pet. ether extract.

Table No.1 Shows the IC₅₀ values of Pet. ether extract of *Vitex leucoxylo*n

Plant Extract	Conc. µg/ml	Absorbance	% inhibition	IC ₅₀ (µg/ml)	R ²
Pet. Ether Extract of <i>Vitex leucoxylo</i> n	62.5	0.561	2.716763	436.7	0.987
	125	0.478333	17.05202		
	250	0.431333	25.20231		
	500	0.244333	57.63006		
	1000	0.128	77.80347		

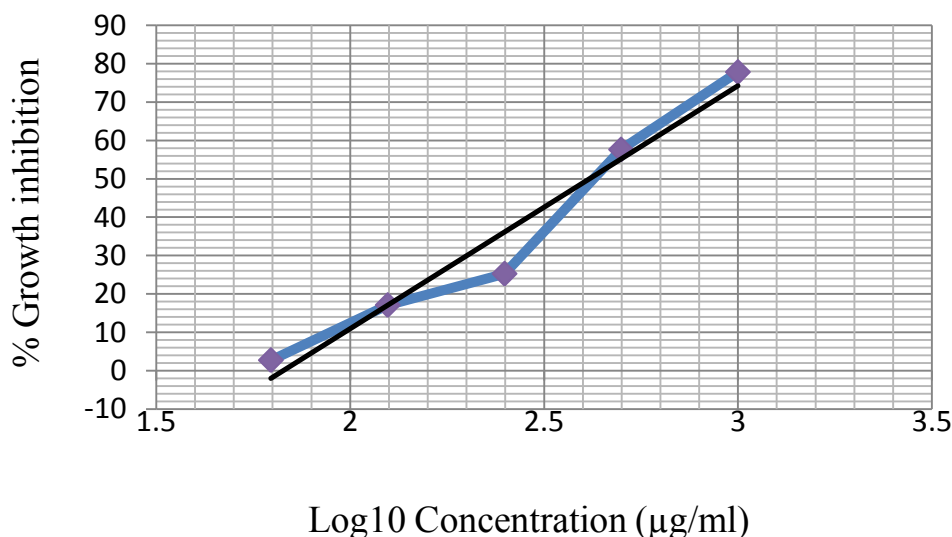
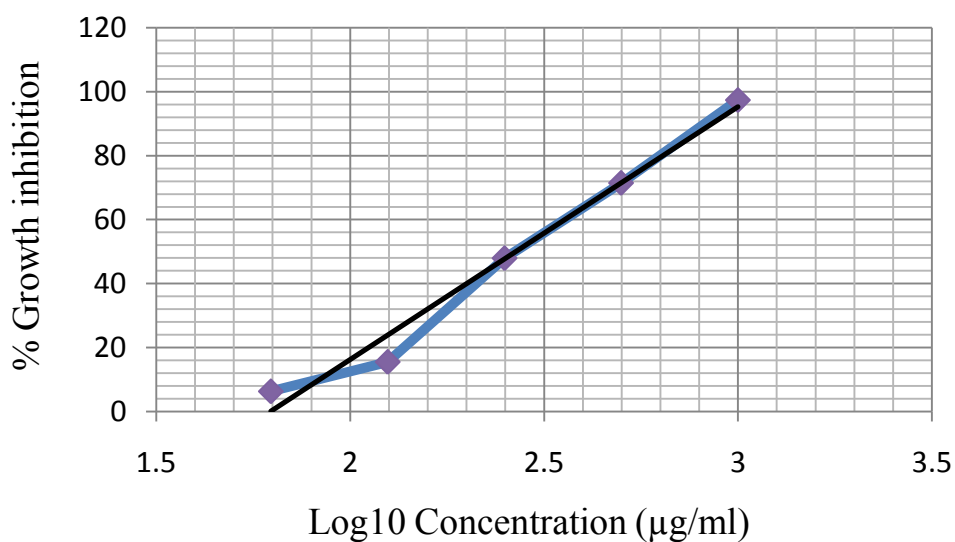


Table No.2 Shows the IC₅₀ values of Chloroform extract of *Vitex leucoxylo*n

Plant Extract	Conc. $\mu\text{g/ml}$	Absorbance	% inhibition	IC ₅₀ ($\mu\text{g/ml}$)	R ²
Chloroform Extract of <i>Vitex leucoxylo</i> n	62.5	0.577333	-0.11561	383	0.9952
	125	0.509667	11.6185		
	250	0.43	25.43353		
	500	0.197667	65.72254		
	1000	0.062333	89.19075		

**Table No.3 Shows the IC₅₀ values of Pet. Ethanol extract of *Vitex leucoxylo*n**

Plant Extract	Conc. $\mu\text{g/ml}$	Absorbance	% inhibition	IC ₅₀ ($\mu\text{g/ml}$)	R ²
Ethanolic Extract of <i>Vitex leucoxylo</i> n	62.5	0.540333	6.300578	277.4	0.9899
	125	0.487667	15.43353		
	250	0.300667	47.86127		
	500	0.165	71.38728		
	1000	0.576667	97.34104		

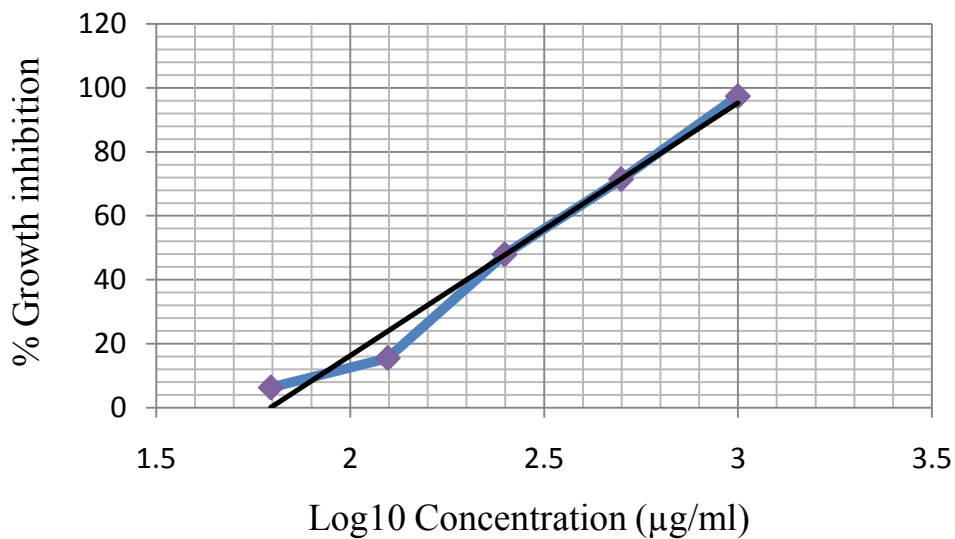
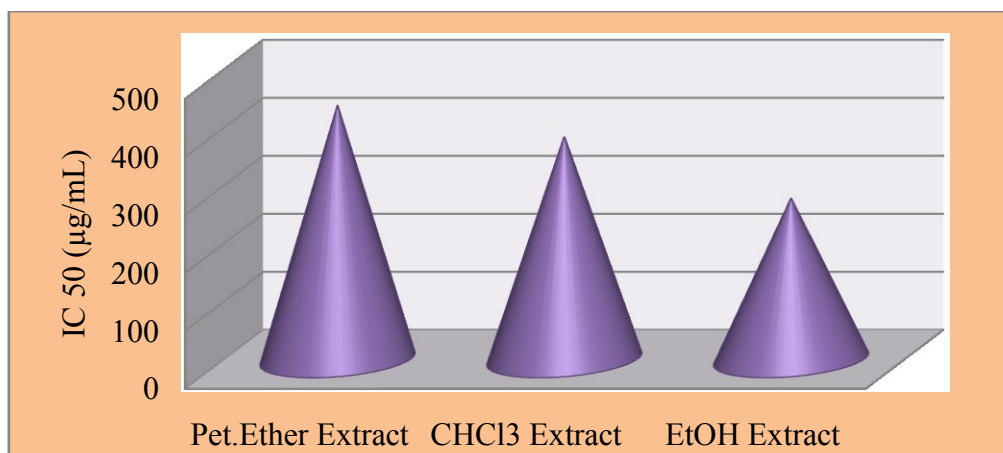
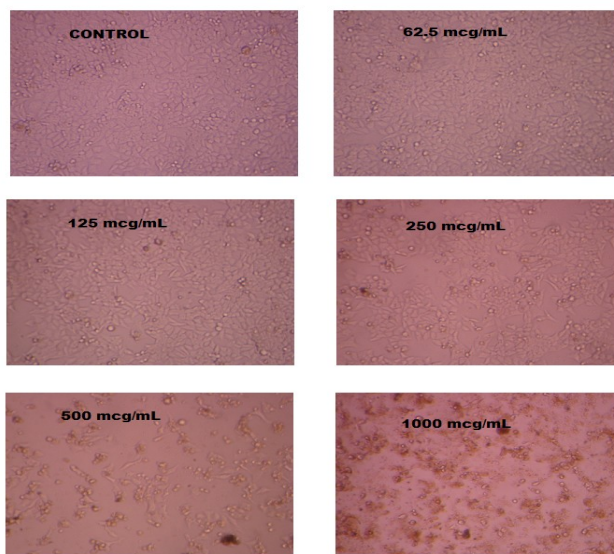


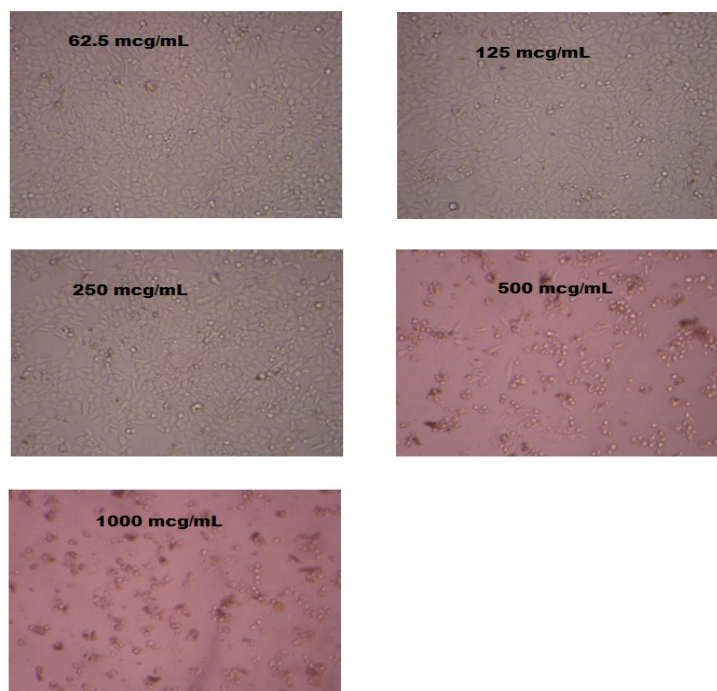
Fig No.4 Comparison of in vitro anti-cancer activity of various extract of leaf of *vitex leucoxylo*



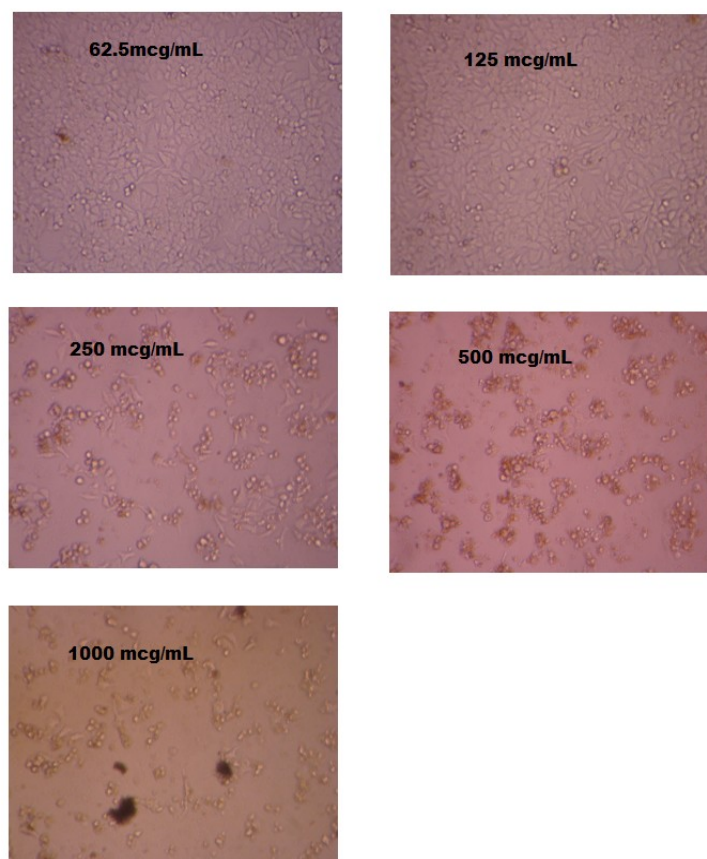
GROWTH INHIBITION OF HELA CELL LINES USING PET.ETHER EXTRACT



GROWTH INHIBITION OF HELA CELL LINES
USING CHLOROFORM EXTRACT



GROWTH INHIBITION OF HELA CELL LINES
USING ETHANOL EXTRACT



BIBLIOGRAPHY

1. Madhava Chetty, K, Sivaji, K. & Tulasi Ro, K. *Flowering plants of chittoor district, Andhra Pradesh, India*. Tirupati: Students offset Printers. 2008. p. 271-272.
2. Monks, A., et al., Feasibility of high flux anti-cancer drug screen using a diverse panel of cultured human tumour cell lines. *Journal of the National Cancer Institute*, 1991. 83, p. 757-766.
3. Mosmann, T., Rapid Colorimetric assay for Cellular growth and Survival : Application to Proliferation and Cytotoxicity assays. *Journal of Immunological Methods*, 1983.65, p. 55-63.
4. O'brien, T.P, Feder, N, and MC Cull, ME., *Polychromatic Staining of Plant Cell Walls by Toluidine Blue-O. Protoplasma*. 1984.59, p. 364-373.
5. Pandey, G, and Sharma, M., Medicinal Plants : Better Remedy for Neoplasms. *Indian Drugs*, 2006. 43(11), p. 869-74.
6. Raj Kapoor, B., et al., Anti-tumour activity and Cytotoxic effect of *Phyllanthus polyphyllus* on Erhlich Ascites Carcinoma and Human Cancer cell. *Journal of Bioscience, Biotechnology, Biochemistry*, 2007.71(9), p. 2177-2183.
7. Rakesh, K., et al., *Herbal Drugs*. New Delhi: Jaypee Brothers, p. 1-3, 337-39.