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IN VITRO ANTI-TUMOUR ACTIVITY OF *VITEX LEUCOXYLON* LINN USING MTT ASSAY METHOD

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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 leucoxylon* by *invitro* method using MTT assay method. These extracts were screened for its cytotoxicity against HeLa cell lines at different concentrations to determine the IC 50 (50% growth inhibition). Ethanol and Chloroform extracts showed more significant effect on the HeLa cell line when compared to Pet. ether extract.

KEYWORDS
Anti-tumour activity, Cytotoxicity, HeLa cell lines, MTT assay and *Vitex leucoxylon.*

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INTRODUCTION
The plant *Vitex leucoxylon* Linn (Family: Verbenaceae) is widely available in Tamilnadu. It is a deciduous tree grows up to 15m tall. The leaves are compound, digitate or rarely trifoliate, minutely pubescent and leaflets 5 (rarely 3). The leaflets are elliptic, apex acute to obtuse, base cuneate-attenuate, margin entire, chartaceous or thinly coaiaceous, glaucous beneath, glabrous, midrib canaliculated above. The leaves are traditionally used for the treatment of leprosy, cancer, emetic, and headache. The present study was carried out to evaluate the *in-vitro* anti tumour activity of various extracts of leaves *Vitex leucoxylon* Linn using MTT assay method.

EXPERIMENTAL METHODS

Plant material

The leaves of plant of *Vitex leucoxylon* 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, Ph.D (Reg.No.PARC/2012/1135). The shade dried plant material was coarsely powdered and used for further studies[1].

Plant Extract

Pet Ether Extract (Table No.1)
Chloroform Extract (Table No.2)
Ethanol Extract (Table No.3).

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 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

MTT assay [2,3]

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.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a haemocytometer and diluted with medium with 5% FBS to give final density of $1x10^5$ 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 37^0C, 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 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 37^0C, 5% CO_2, 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 37^0C for 4hours. The medium with MTT was then flicked off and the formed Formosan 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.

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

Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC50 was determined using Graph Pad Prism software.

RESULTS AND DISCUSSION

The cytotoxicity study was carried out by MTT assay for plant extract of *Vitex leucoxylon* leaves. These extracts were screened for its cytotoxicity against HeLa cell lines at different concentrations to determine the IC 50 (50% growth inhibition) (Figure No.1, 2, 3 and 4). 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 No.5,6, and 7. The IC_{50} 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 R^2 values were 0.987, 0.9952, 0.9899 respectively. Ethanol and Chloroform extracts showed more significant effect on the HeLa cell line when compared to Pet. ether extract.

Comparison of *In Vitro* Anti-Cancer activity of various Extract of Leaf of *Vitex leucoxylon*

Table No.1: Plant Extract of Pet. Ether

Plant Extract	Conc. µg/ml	Absorbance	% inhibition	IC_{50} (µg/ml)	R^2
Pet. Ether Extract of *Vitex leucoxylon*	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: Plant Extract of Chloroform

Plant Extract	Conc. µg/ml	Absorbance	% inhibition	IC_{50} (µg/ml)	R^2
Chloroform Extract of *Vitex leucoxylon*	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 Plant Extract of Ethanol

Plant Extract	Conc. µg/ml	Absorbance	% inhibition	IC_{50} (µg/ml)	R^2
Ethanolic Extract of *Vitex leucoxylon*	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		

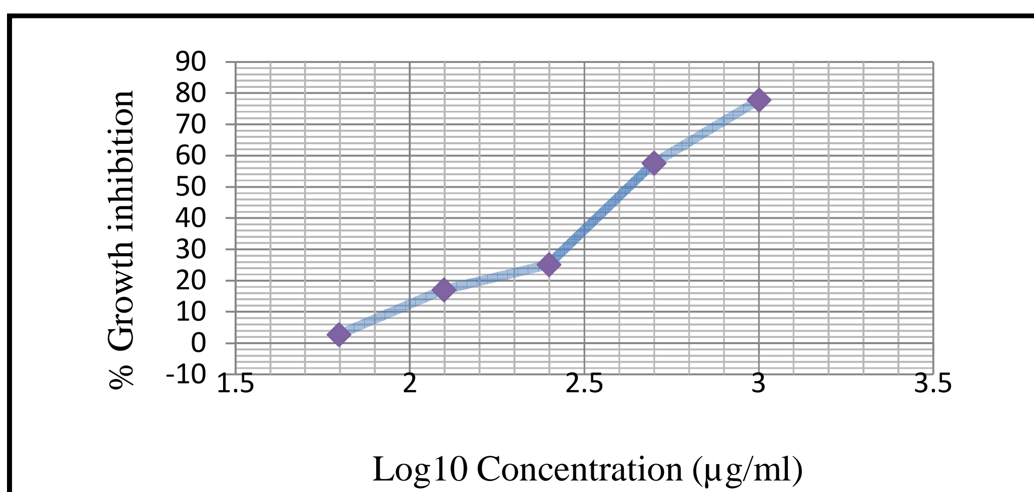

Figure No.1: DRC of Pet. ether extract

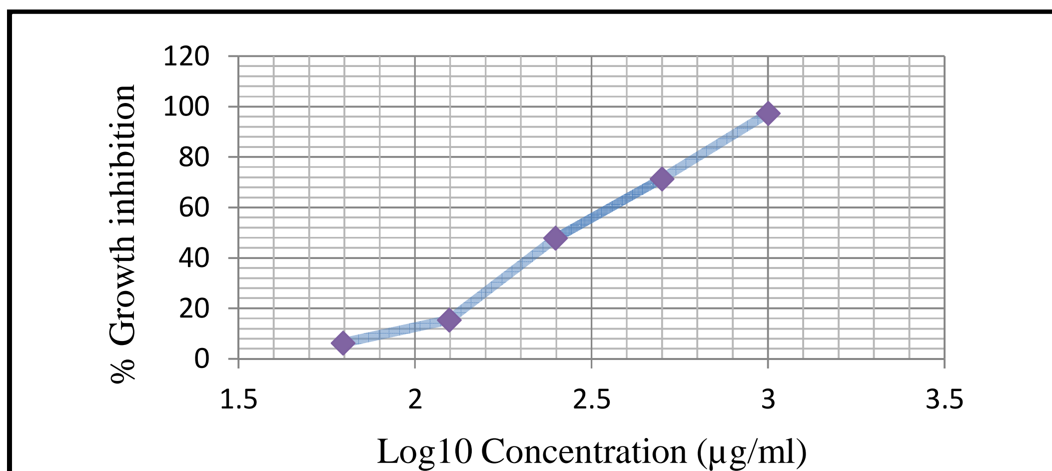

Figure No.2: DRC of chloroform extract

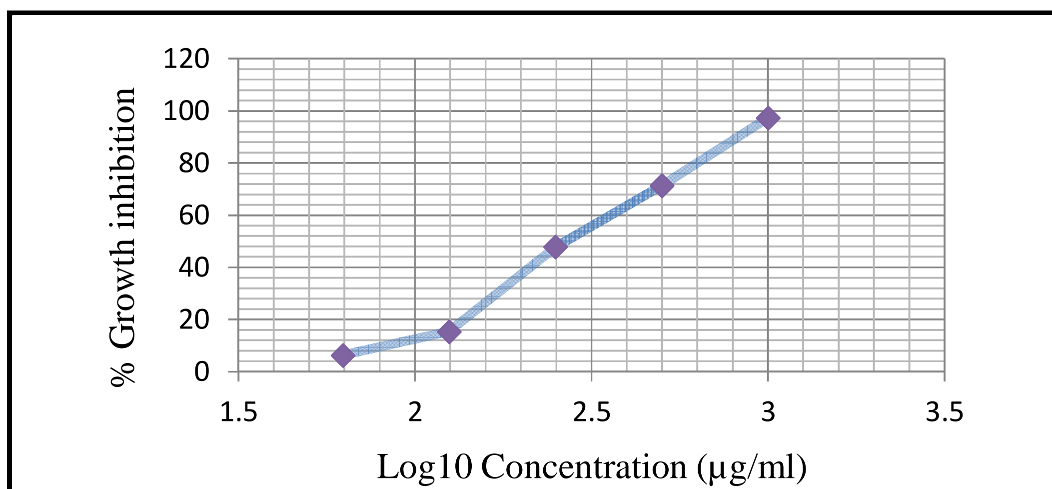

Figure No.3: DRC of ethanol extract

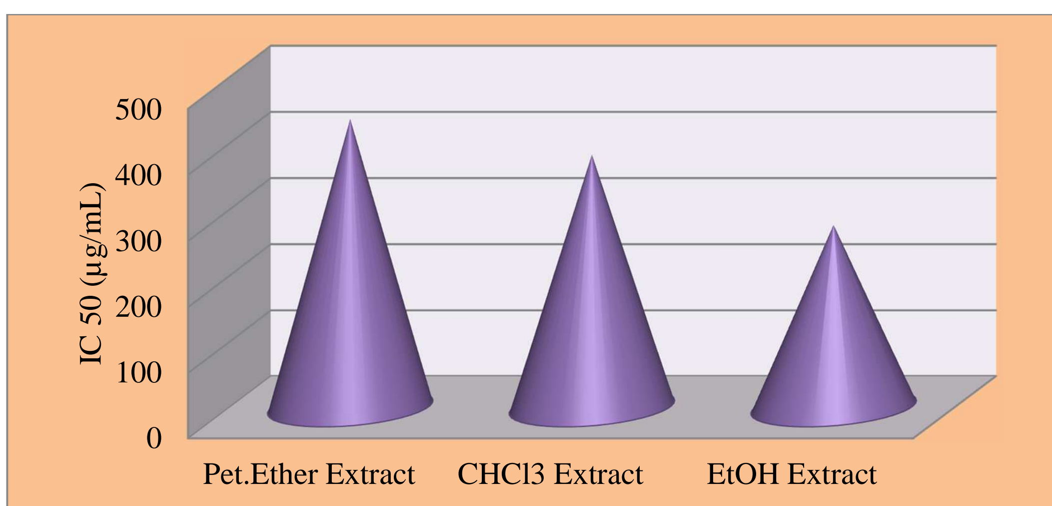

Figure No.4: The IC_{50} value of Pet. Ether, Chloroform and Ethanol extract on the HeLa cell line

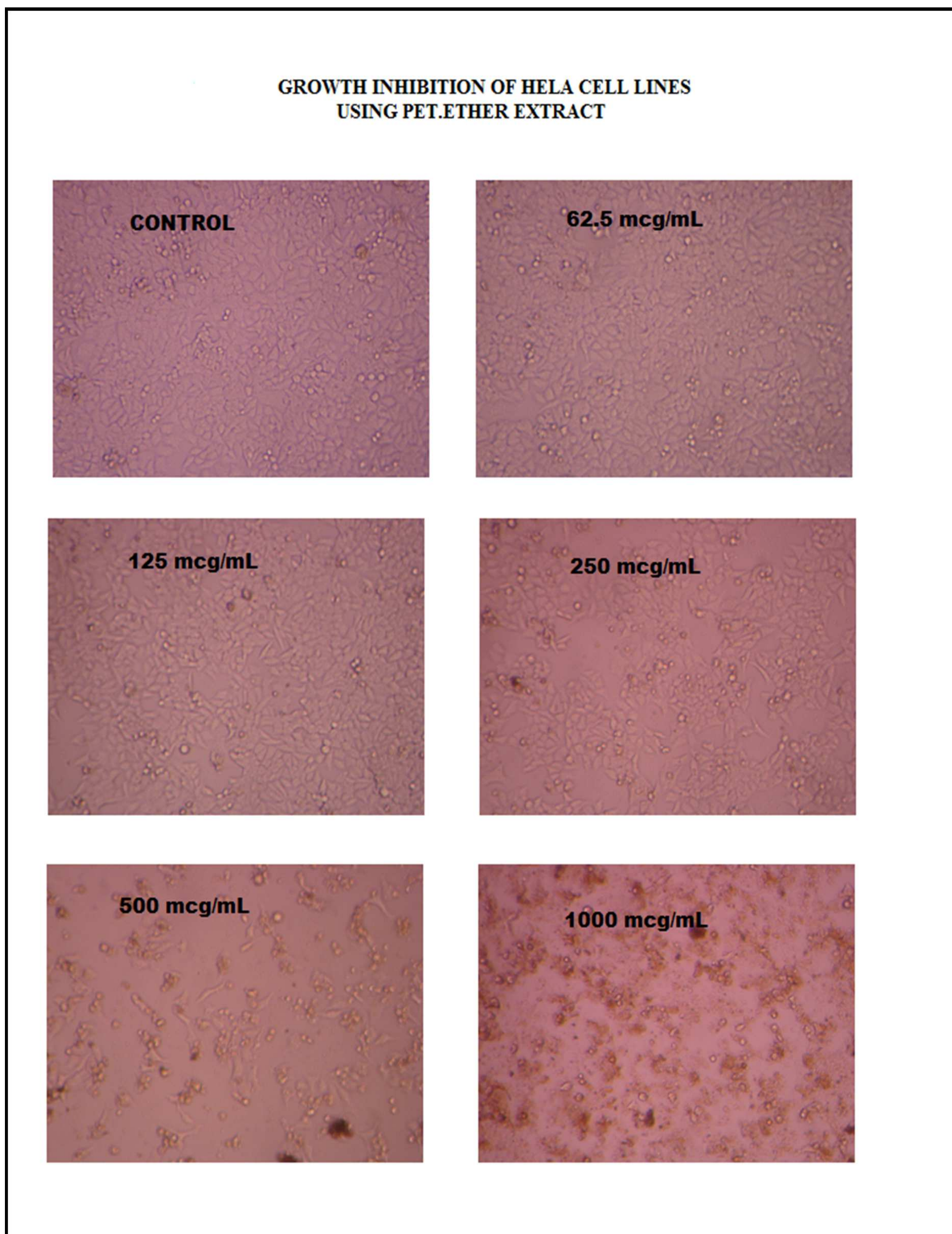

Figure No.5: Growth Inhibition of HeLa cell lines using Pet. Ether Extract

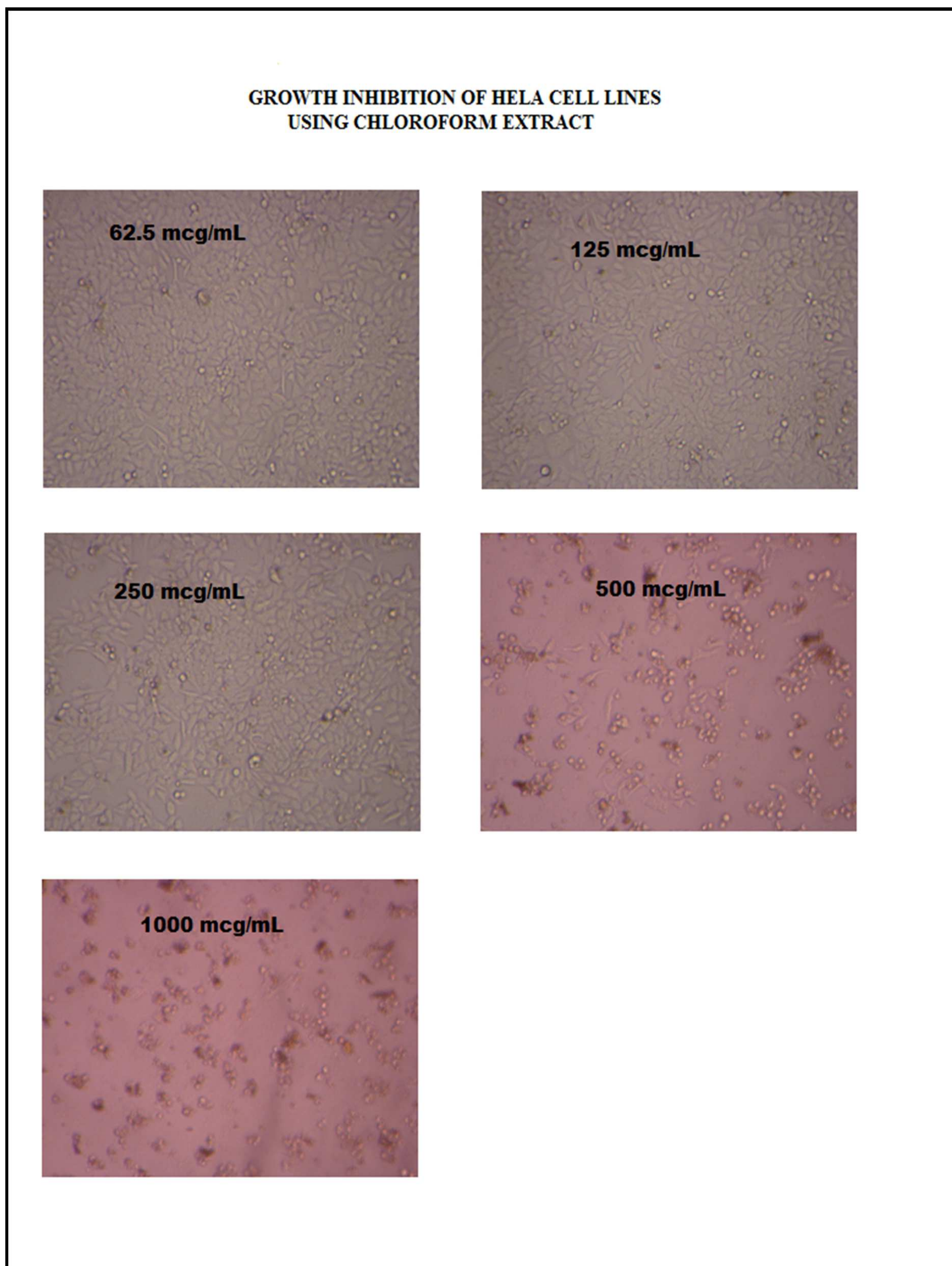

Figure No.6: Growth Inhibition of HeLa cell lines using Chloroform Extract

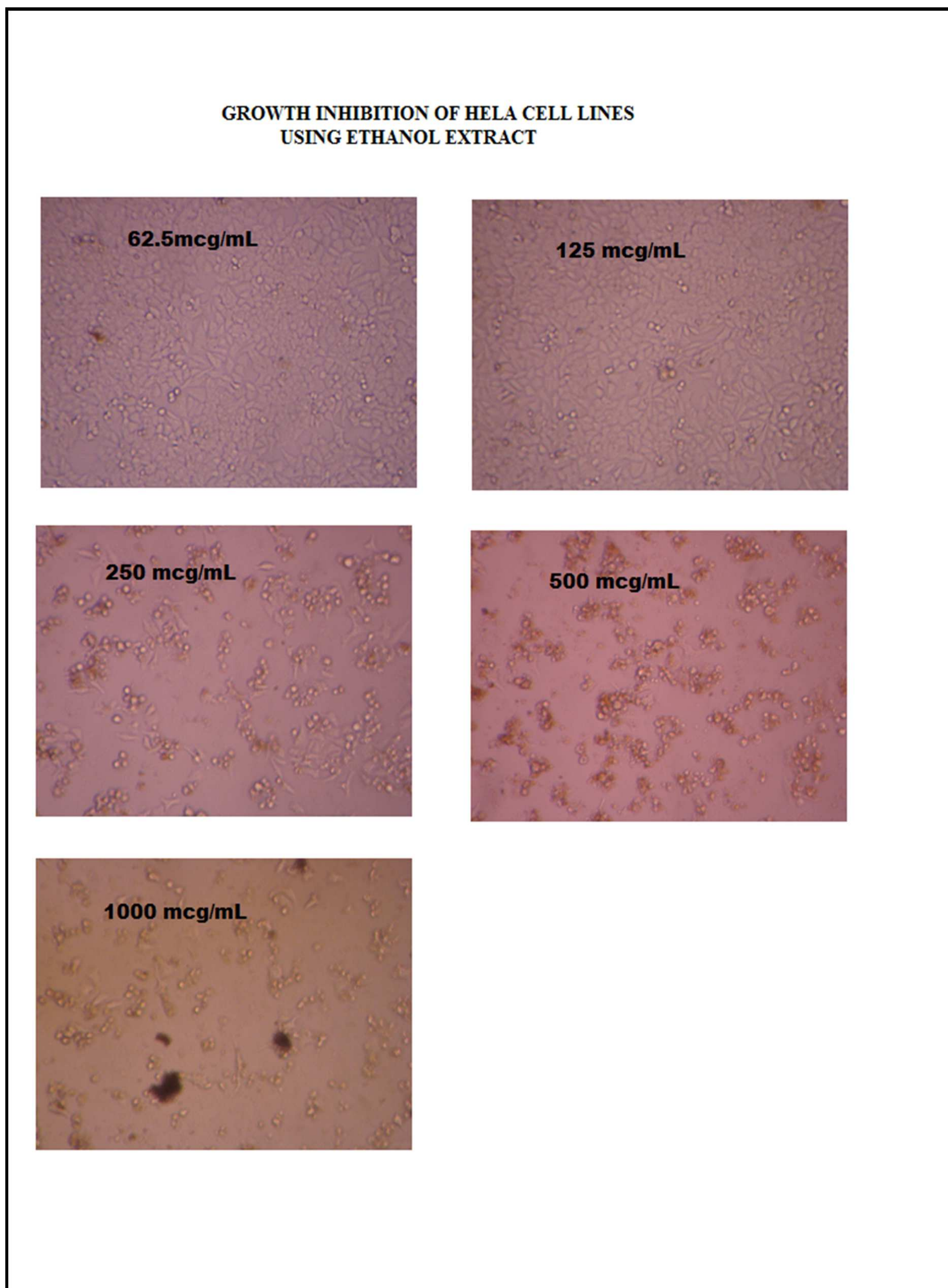

Figure No.7: Growth Inhibition of HeLa cell lines using Ethnol Extract

CONCLUSION

The research work was concluded the Ethanol and Chloroform extracts showed more significant effect on the HeLa cell line when compared to Pet. ether extract.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Madhava Chetty K, Sivaji K and Tulasi Ro K. *Flowering plants of chittoor district, Andhra Pradesh, India*, Tirupati: Students offset Printers, 2008, 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*, 83(11), 1991, 757-766.
3. Mosmann T. Rapid Colorimetric assay for Cellular growth and Survival: Application to Proliferation and Cytotoxicity assays, *Journal of Immunological Methods*, 65(1-2), 1983, 55-63.
4. Obrien T P, Feder N and Cull M C, M E. *Polychromatic Staining of Plant Cell Walls by Toluidine Blue-O. Protoplasma,* 59(4), 1984, 364-373.
5. Pandey G and Sharma M. Medicinal Plants: Better Remedy for Neoplasms, *Indian Drugs*, 43(11), 2006, 869-74.
6. Rajkapoor, *et al.,* Anti-tumour activity and Cytotoxic effect of Phyllanthus polyphyllus on Erhlich Ascites Carcinoma and Human Cancer cell, *Journal of Bioscience, Biotechnology, Biochemistry*, 71(9), 2007, 2177-2183.
7. Rakesh K., *et al., Herbal Drugs*, New Delhi: Jaypee Brothers, 1-3, 337-39.

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