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Research Article

ANTIPROLIFERATIVE ACTIVITY OF *Barleria acuminate* Nees AGAINST IN HUMAN CERVICAL CANCER (HELA) CELLS

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A B S T R A C T

The objective of this study was to examine the cytotoxic effect of *BarleriaacuminataNees* plant in rootand stem extract on cervical cancer (HeLa) cell lines. HeLa cells were cultured in EMEM medium and incubated with different concentrations (12.5, 25, 50, 100 and 200 µg/ml) of ethanol extract of *B. acuminata*. The method used for this research to determine cell viability wasan 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.*B. acuminate* decreased cell viability in malignant cells in a concentration dependent manner. The IC_{50} value of *B. acuminata*extract was found to beroot 166.1µg/mland stem168.2 µg/ml. These data showed ethanolic extract of *B. acuminata*reduces the viability of cervical cell lines and may have potential as anti-cancer compounds.

INTRODUCTION

Cancer is a major health problem in both developed and developing countries. Cancer is one of the leading causes of death worldwide and is characterized by proliferation of abnormal cells (P.C. Nagajyothi *et al*). China, India, and Russia, which share rapidly rising cancer incidence, have cancer mortality rates that are nearly twice as high as in the UK or the USA. Vast geographies, growing economies, aging populations, increasingly westernized lifestyles, relatively disenfranchised subpopulations, serious contamination of the environment, and uncontrolled cancer causing communicable infections have all contributed to its rapid rise in incidence (Goss PE *et al*). Under Indian circumstances cancer could lead to severe social and economic consequences, frequently causing family hardships and societal inequity. In a population of ~1.2 billion, nearly >1 million new cases of cancer are diagnosed every year causing ~600,000e700,000 deaths in 2012 (Mallath MK *et al*).Surgery, chemotherapy, and radiotherapy methods are used in cancer treatment. However, these standard methods are expensive and have side effects with limitations of their use, so there is an urgent need for effective, inexpensive and non-toxic, treatments with minimal side effects that are acceptable by people (P.C. Nagajyothi *et al*).

Cervical cancer is a major cause of death. It is the second most frequent cancer in women worldwide. Human Papilloma virus (HPV) is considered as the etiologic agent of cervical cancer. Epidemiological and biological studies have shown close relationship between HPV infection and cervical cancer development. High risk HPV, such as HPV16 and HPV18, has been detected in 94 - 100% of cervical precancerous lesions and cancer (Castellsagué *et al*., 2006). Though the cervical cancer therapy is in advance, side effects due to the non-specific cytotoxicity of drugs and resistance to treatment represent a great problem in the cervical cancer management. Therefore, development and search of novel and effective anticancer agents, which in addition should overcome resistance, have become very important issues (Cameron and Bell, 2004). Natural compounds have provided many effective anticancer agents in current use. Currently, over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources or are related to them (Newman and Gragg, 2007). The use of plants or plants products, traditionally, as antiviral agents is relatively wider than their use in modern medicine. Some antiviral substances have so far been isolated from higher plants, algae and lichens (Abonyi *et al*., 2009). The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity (Mohammad S, 2006). Over 3000 species of plants with antitumour properties have been reported (Hartwell JL, 1982).Natural products have been regarded as important sources of potential chemotherapeutic agents and many anticancer drugs have originated from natural sources (Tan G *et al*, 2006).

MATERIALS AND METHODS

Preparation of Leaf Extract

*BarleriaacuminataNees*rootand stem were collected from Pachaimalai, Tiruchirappalli District, Tamilnadu, Indiaand were air dried and grounded to fine powder. Then the powdered material was extracted with ethanol by using soxhlet apparatus. The solvent was removed by evaporation

and extract was concentrated by using vacuum rotator evaporator.

Cell Line

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). The 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.

Cell Treatment Procedure

The monolayer cells were detached with trypsin-ethylenediaminetetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 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 h the cells were treated with serial concentrations of the test samples. They were initially dissolved or dispersed in dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT Assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (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 48 h 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 % cell inhibition was determined using the following formula: Percentage cell inhibition = 100 Abs (Sample) / Abs (Control) x 100.

Statistical Analysis

Non-linear regression graph was plotted between % cell inhibition and log10 concentration and IC50 was determined using GraphPad Prism software.

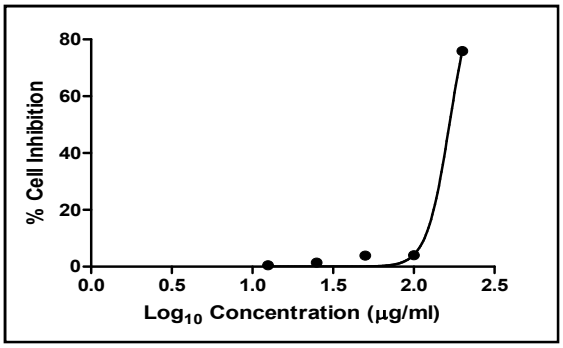


Fig.1 Percentage growth inhibition of *B. acuminata*root against HeLa cell line

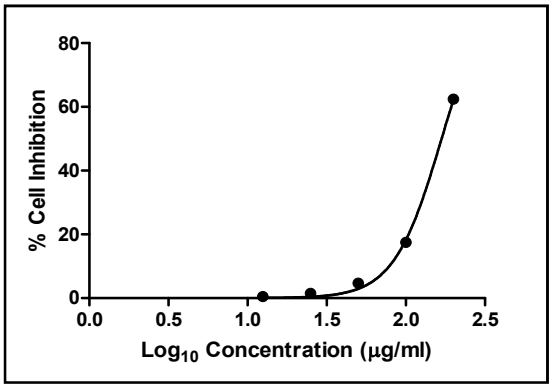


Fig.2 Percentage growth inhibition of *B. acuminata*stemagainst HeLa cell line

RESULTS AND DISCUSSION

The results for cell growth inhibition by the extract against HeLa cell lines for various concentrations is shown in table 1. In the present study HeLa cells showed growth inhibition in a dose dependent manner when treated with *B. acuminata*extract at concentrations ranging from 12.5µg to 200µg. The percentage of dead cells for each concentration was found to be root0.49, 1.43, 3.87, 4.05, 75.8 and stem 0.49, 1.49, 4.68, 17.4, 62.46.The 50% cytotoxic effect (IC50) of *B. acuminata*extract was found to be root 166.1µg/mland stem 168.2 µg/ml(Table I& 2 and Figs. 1,2 & 3,4). The utility of cell lines acquired from tumors allows the investigation of tumor cells in a simplified and controlled environment (Arya *et al*., 2011). There are specific advantages and disadvantages to exploit cancer cell lines over animal models. These then dictate the nature of the experiment that can be organised. In the last few decades, studies with cell lines can serve as an initial screen for agents that might regulate drug resistance. *B. acuminata*has been widely studied for its antioxidant activity. Now-a-days, after this antioxidant was found to offer protection against the occurrence of cancer activity (Ledy*et al*., 2012). In the present study the HeLa cell lines are used as a model for studying cervical cancer. Several mechanisms of action were detected in HeLa cells. The IC50 of extract on cell line less than 100 µg / ml is categorized as a potential cytotoxic substance (Spavieri*et al*., 2010). In the present study, ethanol extract of *B. acuminata*was found to be moderately cytotoxic towards human HeLa in MTT assay and the concentration required for 50% cell death was found to beroot 166.1µg/mland stem 168.2 µg/ml. Hence present study shows the efficacy of *B. acuminata* for the antiproliferation of HeLa cells thus suggesting protection against cervical cancer.

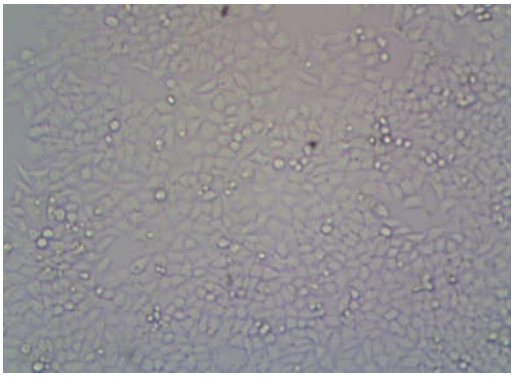
In summary, the present study demonstrated that ethanol extract of *B. acuminata*is a potent anti-cancer compound with an IC_{50} of root 166.1µg/mland stem 168.2 µg/ml inducing growth inhibition in the human cervical cancer cells.

Table.1 In vitro Cytotoxicity Root of *B. acuminata* on HeLa cell lines

Plant Extract	Concentration (µg / ml)	% inhibition	IC50 µg / ml	R2
B. acuminata Root	12.5	0.499688	**166.1 µg/ml**	**0.9961**
	25	1.436602		
	50	3.87258		
	100	4.059963		
	200	75.89007		

Table.2 In vitro Cytotoxicity Stem of *B. acuminata* on HeLa cell lines

Plant Extract	Concentration (µg / ml)	% inhibition	IC50 µg / ml	R2
B. acuminata Stem	12.5	0.499688	**168.2 µg/ml**	**0.9981**
	25	1.499063		
	50	4.684572		
	100	17.48907		
	200	62.46096		



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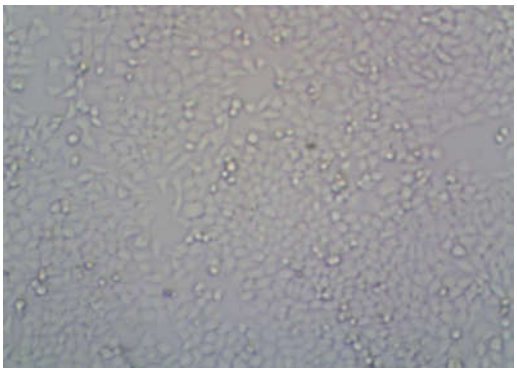

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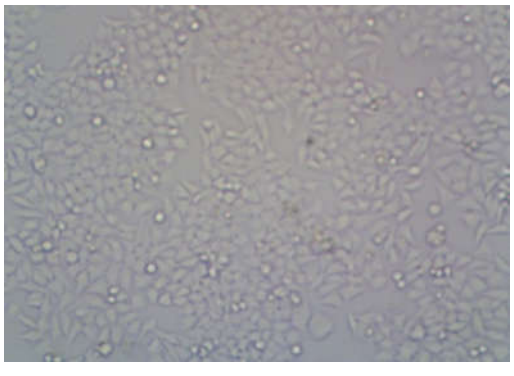

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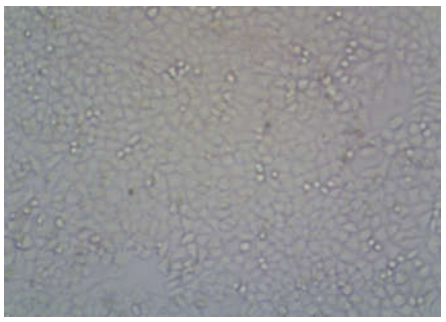

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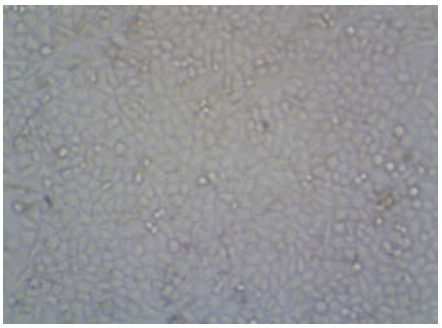

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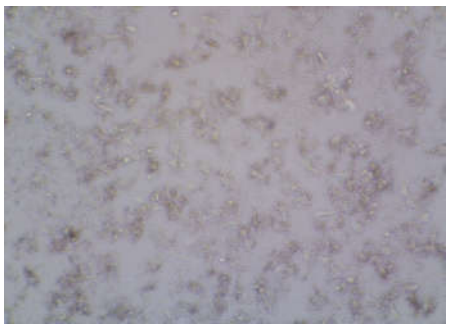

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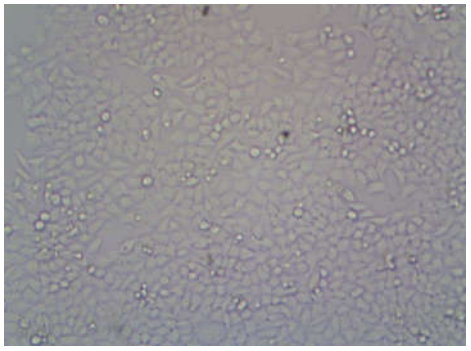
A - Control ; B - 12.5 g ; C - 25 g ; D - 50 g ; E - 100 g ; F - 200 g

Fig.3 Proliferation of HeLa cells treated with Rootof *B. acuminata*



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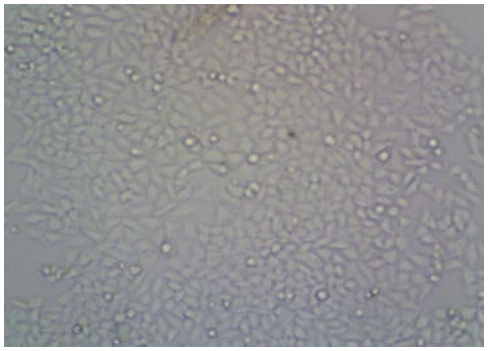

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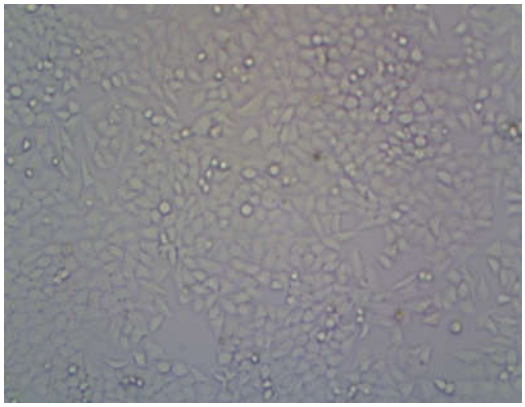

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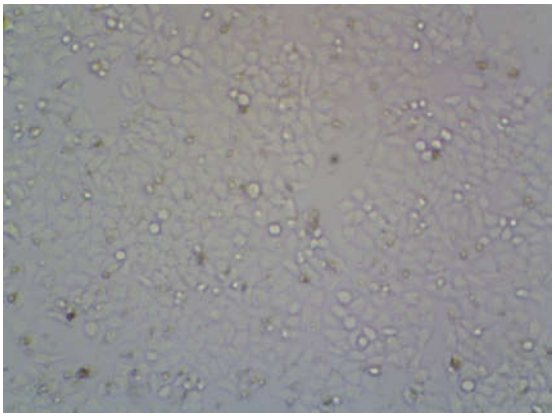

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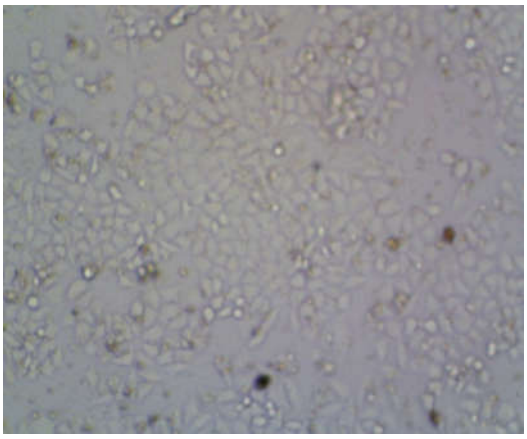

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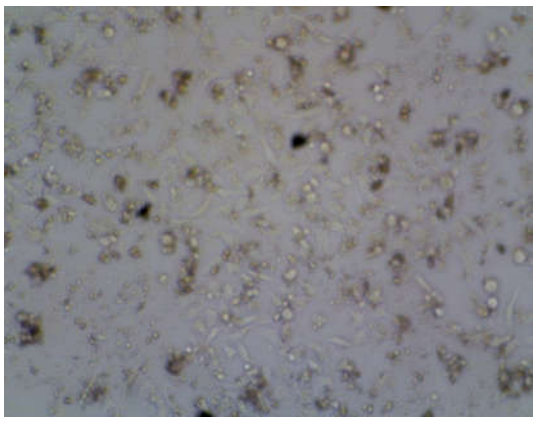

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A - Control ; B - 12.5 g ; C - 25 g ; D - 50 g ; E - 100 g ; F - 200 g

Fig.4 Proliferation of HeLa cells treated with stemof *B. acuminata*

References

P.C. Nagajyothi, P. Muthuraman, T.V.M. Sreekanth, Doo Hwan Kim, Jaesool Shim, 2016. Green synthesis: In-vitro anticancer activity of copper oxide nanoparticles against human cervical carcinoma cells. *Arabian Journal of Chemistry*.

Goss PE, Strasser-Weippl K, Lee-Bychkovsky BL, *et al*. Challenges to effective cancer control in China, India, and Russia. *Lancet Oncol.* 2014;15:489e538.

Mallath MK, Taylor DG, Badwe RA, *et al*. The growing burden of cancer in India: epidemiology and social context. *Lancet Oncol*. 2014;15:e205ee212.

Castellsagué X, Diaz M, de Sanjosé S, Munoz N, Herrero R, Franceschi S, Peeling RW, Ashley R, Smith JS, Snijiders PJ, Meijer CJ, Bosch FX (2006). Woldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: implications for screening and prevention. *J. Natl. Cancer Inst*. 98: 303-315.

Cameron D, Bell R (2004). Optimizing treatment for patients with breast cancer. Semin. *Oncol.* 31: 4–5.

Newman DJ, Gragg GM (2007). Natural Products as Sources of New Drugs over the Last 25 Years. *J. Nat. Pro*d. 70: 461-477.

Abonyi DO, Adikwu MU, Esimone CO, Ibezim EC (2009). Plants as sources of antiviral agents. *Afr. J. Biotechnol*. 8: 3989-3994.

Mohammad S. Anticancer agents from medicinal plants. *Bangladesh J Pharmacol* 2006; 1: 35-41.

Hartwell JL. Plants used against cancer. A survey. Quarterman Publications, Lawrence; 1982.

Tan G, Gyllenhaal C, Soejarto D.D. Biodiversity as a source of anticancer drugs. *Curr Drug Targets* 2006; 7: 265-277.

Arya, V., C.P. Kashyap, T. Bandana, S. Shiksha, K. Sweta, P. Verma and Swati Sharma, 2011. Human cancer cell lines A brief communication*, J. Chem. Pharm. Res*. 3(6):514-520.

Spavieri, J., A. Allmendinger, M. Kaiser, R. Casey, S. Hingley-Wilson and Lalvani, A., 2010. Antimycobacterial, antiprotozoan and cytotoxic potential of twenty-one brown algae (Phaeophyceae) from British and Irish waters. *Phytother. Res*. 24: 1724-29.