

**EVALUATION OF THE *INVITRO* ANTI CANCER POTENTIAL OF
*ANOGEISSUS ACUMINATE******Raghu S., Sankar V., Kothai R. and Arul B.**

Department of Pharmacology, Vinayakamission's College of Pharmacy, Vinayakamissions
University, Salem-636008.

Article Received on
30 June 2017,

Revised on 21 July 2017,
Accepted on 11 August 2017

DOI: 10.20959/wjpr20179-9312

Corresponding Author*Raghu S.**

Department of
Pharmacology,
Vinayakamission's College
of Pharmacy,
Vinayakamissions
University, Salem-636008.

ABSTRACT

The present study was aimed to investigating the anti cancer activity of the ethanolic extract of *Anogeissus acuminata* was studied by using different *invitro* methods Previously the plant has not been investigated for anticancer activity to any greater extent of the plant possessed very good antioxidant properties as evident in the previous studies, it is anticipated that it may possess potential anticancer activity also. The effects of alcoholic extracts of *Anogeissus acuminata* were tested on HELA i.e., epithelial human cancer cell lines in *invitro* by Tryphan blue exclusion assay and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) Assay method. The ethanolic extract of the plant 640µg/ml had shown potent activity in MTT and Tryphan blue exclusion assay. The findings suggest that the ethanol

extract of *Anogeissus acuminata* is a effective anticancer properties, augmenting its therapeutic value.

KEYWORDS: *Anogeissus acuminata*, Hela, Tryphan blue, MTT, Anticancer.

INTRODUCTION

Cancer is one of the major human diseases and causes large suffering and economic loss world-wide. Chemotherapy is one of the methods of treating cancer. However the chemotherapeutic drugs are highly toxic and have devastating side effects. Various new strategies are being developed to control and treat several human cancers.^[1] Still the currently available therapies for cancer have many lacunae which affect the patient's health severely in the form of side effects. That more than 50% of all modern drugs in clinical use are of natural products, many of which have been recognized to have the ability to include apoptosis in

various cancer cells of human originals, there is an urgent need to develop much effective and less toxic drugs.^[2] The utility of cell lines acquired from tumors allows the investigation of tumor cells in a simplified and controlled environment. This is a useful tool for primary screening of cancer. The HeLa was the first human cell line established in culture and has since become the most widely used human cell line in biological research.^[3] *Anogeissus acuminata* is known as Button tree. It is a deciduous tree with a narrow crown; it can grow up to 40 meters tall the long, straight bole is unbuttressed and can be 100cm in diameter widely used in skin diseases like eczema, dermatitis, skin ulcers and anti-inflammatory and analgesic activity. The present study will be discussed about invitro anticancer effect of the plant.^[4,5]

MATERIALS AND METHODS

Plant material

The plant *Anogeissus acuminata* was collected from Tirupathi hills, Andhra Pradesh. The plant was taxonomically identified and authenticated by the Botanist Dr. V. Chelladurai, The authenticated plant material was used for the preparation of extracts.

Preparation of plant extract^[6]

The leaves of this plant were dried under shade at 27-30°C for 15-30 days, after which the leaves of the plant were chopped and grounded into coarse powder. The powder (400 g) was extracted with ethanol (1500 ml) overnight, at room temperature with constant stirring. The extraction was carried out by continuous hot percolation using soxhlet apparatus. The extract was filtered and the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator.

The yield (w/w) of the crude extract was found to be 83.08%. Phytoconstituents present in the various extracts were identified by chemical tests shows high phenolic and flavonoids contents.

Drugs and chemicals

All the drugs and chemicals used in the study were obtained commercially and were of analytical grade.

Cell line

The Cancer cell line HeLa was purchased from National Centre for Cell Science (NCCS), Pune, India.

***In-Vitro* Cytotoxicity (Trypan blue method)^[7,8]**

Short term in-vitro cytotoxicity was assessed using HELA cancer cell lines cells by incubating different concentrations of the *Anogeissus acuminata* at 37°C for 3 hours. The tumor cells were aspirated from peritoneal cavity of tumor bearing mice using an insulin syringe and transferred to a test tube containing isotonic saline. The cells were then washed in normal saline and cell number was determined using a Haemocytometer and adjusted at 10×10^6 cells/ml. For the cytotoxicity assay, different concentrations of the extracts (25-1600 µg/ml) were added to each tubes and the final volume was adjusted to one ml with normal saline. Control tubes were kept with the saline, tumor cells and without the drugs.

All the tubes were incubated at 37c for 3hours. After incubation 0.1ml of 0.4% trypan blue dye in isotonic saline was added to each tube and the number of viable (unstained) and dead (stained) cells were counted using haemocytometer.

$$\% \text{ Dead cells} = \frac{\text{Total cells counted} - \text{total viable cells}}{\text{Total cells counted}} \times 100$$

Cytotoxic Studies -MTT Assay^[9,10]

This is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent (eg. isopropanol) and the released, solubilized formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. FHC and A-549 cells were treated with *Anogeissus acuminata* and 5-FU (1 - 0.039µM). Cell viability was determined at 24 h based on MTT assay. Briefly, the cells were seeded in a 96-well plate at a density of 4×10^3 cells/well and allowed to adhere overnight. After removing the medium, 200 µL fresh medium per well, containing 10 mmol/L HEPES (pH 7.4), was then added. Then, 50 µL MTT was added to the wells and the plate incubated for 2 - 4 h at 37°C in the dark. The medium was removed, and 200 µL DMSO and 25 µL Sorensen's glycine buffer were added to the wells. Absorbance was measured using an ELISA plate reader at 570 nm.

RESLUTS AND DISCUSSION

Trypan Blue Exclusion Assay

Trypan Blue is a blue acid dye that has two azo chromophore groups. Trypan Blue is an essential dye, used in estimating the number of viable/dead cells present in a population. Trypan blue is a vital stain used to selectively colour the dead tissues. It is a diazo dye. Live cells or tissues with intact cell membranes are not coloured as trypan blue is not absorbed; however, it traverses the membrane in a dead cell and are shown as a distinctive blue colour under a microscope. Since live cells are excluded from staining, this staining method is also described as a dye exclusion method. Staining facilitates the visualization of cell morphology

The *in vitro* cell viability assay by trypan blue exclusion method was carried out to evaluate the antitumor potential of EAC against HELA cell lines. The antitumor effect of EAC against HELA cell line produced a concentration dependent cytotoxic effect which was indicated by the increase in number of dead cells with increasing concentrations of EAC. The 10 µg concentration of EAC showed 15.8% of dead cells where as in higher concentration of 640 µg EAC 91.52% of dead cells.

The results showed that HELA tumor cell proliferation was significantly inhibited by EAC, with an ED₅₀ value i.e 50% of tumor cell death at 400µg respectively and were shown in Table no. 1, Figure no. 1.

Table No 1: Effect of ethanolic extract of leaves of *EAC* on HELA Cell lines by Tryphan blue method.

S.no	Concentration µg	Cell viability		% of cell death
		Live cells	Dead cells	
1	10	199	35	14.95
2.	20	190	50	20.83
3.	40	180	85	32.07
4	80	140	110	44.0
5	160	100	155	60.78
6	320	45	182	80.17
7	640	25	236	90.42

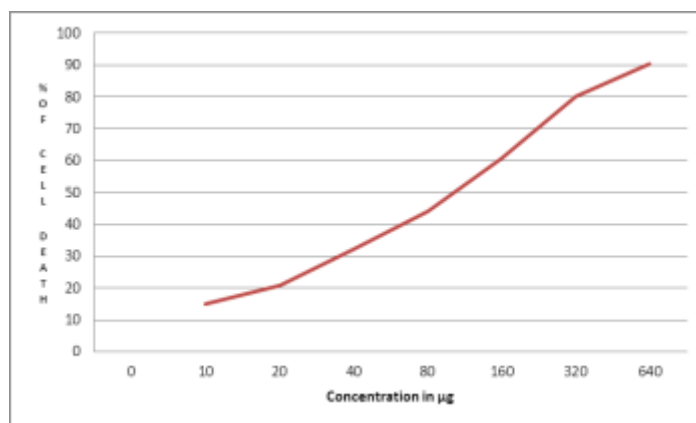


Fig no 1: Shows the result of the HELA cell by Trypan blue method.

MTT ASSAY

The percentage of survived cells was calculated by measuring the absorbance of respective incubated cells in the 96 wells plate. The effect of the extracts on HELA cell lines is significant and comparable to the standard drug Etoposide. The extract have shown the activity even at the lowest concentration of 10 µg/mL. The extract have shown concentration dependent activity. The extracts have Shown are in Table no. 2, Figure no.2.

Table No 2: Effect of ethanolic extract of leaves of *EAC* on HELA cell lines by MTT assay.

S.no	Concentration µg/ml	% cell viability
1	10	95
2	20	78
3	40	70
4	80	58
5	160	38
6	320	18
7	640	0

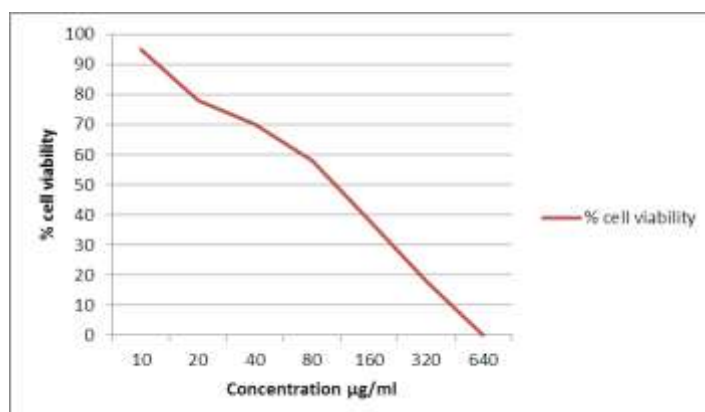


Fig no 2: Effect of ethanolic extract of leaves of *EAC* on HELA cell by MTT assay.

CONCLUSION

Based on the results obtained, *Anogeissus acuminata* was showed potent anti cancer activity not remarkably different than reference compound. Major anticancer component seems to be phenolic and flavonoids. The oxidative stress is an important factor that affects the release and suppress the cancer cell viability. The plant extract produce remarkable effect but the treatment remains unclear and warrants further investigation.

REFERENCES

1. Yadav, B. et al. "In Vitro Anticancer Activity of the Root, Stem and Leaves of *Withania Somnifera* against Various Human Cancer Cell Lines." *Indian Journal of Pharmaceutical Sciences*, 2010; 72(5): 659–663. PMC. Web. 17 June 2017.
2. Chong FW, et al was reported Expression of Transforming Growth factor- β and determination of Apoptotic Index in histopathological sections for assessment of the effects of Apigenin (4',5',7'- trihydroxyflavone)on Cyclosporine A induced renal damage. *Malaysian Journal of Pathology*, 2009; 31(1): 35–43.
3. Kashyap C.P. et al was reviewed. "Human cancer cell lines- A brief communication". *Journal of Chemical and Pharmaceutical Research*, 2007; 113: 115–124.
4. Sankar V. et.al. was reported. Evaluation of the Invitro Antioxidant Effect of *Anogeissus Acuminata*. *World Journal of Pharmaceutical Research*, 2016; 6(5): 1136-11416.
5. Singh and Jialal trees of laos and vietnam: a field guide to 100 economically or ecologically important species in blumea in *Journal of biodiversity, evolution and biogeography of plants*, 2004; 49(2-3): 201, 349(149).
6. Dr. S. Khadabadi, Dr. S. I. Derore, B. ABaviskar. *Experimental phytopharmacognosy a comprehensive guide*; Nirali prakashan, 1.7-1.8.
7. Ashwini s et.al. was reported . Seaweed extracts exhibit anticancer activity against hela cell lines. In *international journal of current pharmaceutical research*, 2010; 1: 114-117.
8. Asirvatham R, et al. was studied. "In Vitro Antioxidant and Anticancer Activity Studies on *Drosera Indica* L. (Droseraceae)." *Advanced Pharmaceutical Bulletin*, 2013; 3(1): 115-120.
9. Ahmadian, Somaieh et al. was reported. "Cellular Toxicity of Nanogenomedicine in MCF-7 Cell Line: MTT Assay." *Journal of Visualized Experiments*, 2009; 26: 1191.
10. Mosmann T. was studied. Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assays. in *Journal of Immunological Methods*, 1983; (1-2): 55-63.