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CYTOTOXIC SCREENING OF *WITHANIA SOMNIFERA, CAPSICUM ANNUUM AND TAMARINDUS INDICA ON* MCF7, A549 AND PA1 CANCER CELL LINE

Rajeev Nema*[1], Sarita Khare[1], Alka Pradhan[1], Amit Deshpande[2]

1. Sarojini Naidu Government Girls Post Graduate (Autonomous) College, Shivaji Nagar, Bhopal - 462016 (M.P.)
2. Deshpande Lab. and Pvt. Ltd., Bhopal, MP. India.

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For Correspondence:

Rajeev Nema

Sarojini Naidu Government Girls Post Graduate (Autonomous) College, Shivaji Nagar, Bhopal - 462016 (M.P.)

E-mail:

rrsht.nema@gmail.com

ABSTRACT

The present study aimed in the direction of three different medicinal plants (hydro alcoholics extract*) to evaluate anticancer activity. Withania Somnifera Capsicum annuum and Tamarindus indica* is the significant medicinal plants. The most important purpose of this study is to evaluate cytotoxicity of these medicinal plants with facilitate of MTT assay. The study of angiogenesis is making a deep impact on the biological and medical world. Concentrations are prepared of each plant extracts which are 100 µg/ml, 10 µg/ml , 0.1 µg/ml, 0.01 µg/ml and 5-10×10 3 cells/ml are taken into each well which are exposed to different Concentrations of plant extracts for 96 hr and then treated with MTT. For MTT absorbance in use at 570 nm. From IC50 values of MTT assay of *Tamarindus indica*, *Capsicum annuum* and *Withania Somnifera* for MCF7, A549 and PA1 cancer cell lines, from this it may conclude that *Withania Somnifera* shows effective cytotoxicity than *Tamarindus indica* and *Capsicum annuum* for respective cell lines.

INTRODUCTION

Ayurveda is the oldest existing complete medical system in the world. Its origins go back nearly 5000 years. Some Medicinal plants have therapeutic potential due to the occurrence of natural antioxidants functioning as reducing agents, free radical scavengers and quenchers of singlet oxygen. Majority of their antioxidant activity is due to bioactive compounds viz. flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins and isocatechins. In the present time herbal products are considered to be symbols of protection in comparison to the synthetic product that are regarded as unsafe to human life and environment. Although herbs had been priced for their medicinal importance. And now the major challenging and exclusive activity of pharmaceutical industry is drug improvement and discovery. The discovery of new drugs and their development into commercial products takes place across the broad scope of the pharmaceutical industry and research institutes Attempts are underway to work out the therapeutic and anti-neoplastic properties of medicinal plants (Ahmad et al., 1998; Datta et al., 1998; Abo et al., 2000; Graf, 2000; Ankli, 2002; Neto, 2002). Consequently, herbal medicines have received much attention as substitute anticancer drugs.

MEDICINAL PLANTS AS AN ALTERNATIVE TREATMENT TO CANCER

Plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities owing to their enormous propensity, which synthesize a variety of structurally diverse bioactive compounds (Kim *et al.,* 2005; Indap *et al.,* 2006). The rich and diverse plant sources of India are likely to provide effective anticancer agents. Medicinal plants can reduce or minimize the toxic side effect of chemotherapy and radiation treatment by reinforcing their cancer killing action. Some of the plants, selected for the present study, include: *Withania Somnifera Capsicum annuum and Tamarindus indica.*

MATERIAL AND METHODS REAGENTS

Alcohol 70%, 100% Alcohol, MEM media (Minimal Essential Media) (Eagle H 1959), Trypsin (Cole RJ and Paul J, 1966) ,MTT (3-(4,5- Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide, a tetrazole) (Mosmann T 1983), Distilled Water, Dimethyl sulphoxide (DMSO) (Lovelock, JE Bishop MH 1959), etc.

REQUIREMENTS

Laminar air flow, Autoclave, N2 liquid container, CO2 incubator, Inverted microscope, Filtration assembly, Hemocytometer, Centrifuge machine, Micropipette.

PREPARATION OF PLANT EXTRACT

Weighed 25 gm of drug and transferred it into container containing menstruum as well as covered it with help of cotton or other closure which is suitable one. Kept it for sufficient duration of time. Shaking of drug during maceration is essential in order to replace the saturated layers around the drug with fresh menstruum. After straining, the marc was pressed in filter press, hydraulic press or hand press. Subsequently went on behalf of concentration of drug.

ISOLATION OF HUMAN CANCER CELLS

Human cancer cells are isolated from the patients and characterized at cellular and molecular levels. Isolated cells are cultivated in specialized mediums and specialized incubators to provide them physiological conditions required for the growth (Satyanarayan U, and Fershney 2000).

CELL LINE

The sub culturing of the primary culture gives rice to cell lines. The term continuous cell line implies the indefinite development of the cell in the successive sub culturing. On the other hand, finite cell lines symbolize the death of cell after several subcultures. The considered cell lines are **MCF7 (**Soule HD 1973 and Giard DJ et al 1972) **(**barest cancer), **A549 (**lung cancer) and **PA1** (ovary cancer).

ASSAY PERFORMED (Soule HD 1973 and Shapiro HM 1988)

MTT ASSAY METHOD

Laminar air flow was prepared. Dilutions of concentration 100 µg/ml, 10 µg/ml, 1 µg/ml, 0.1 µg/ml, 0.01 µg/ml from stock solution (test drug +DMSO) having concentration 10mg/ml are done. Then normal count on haemocytometer before seeding the cells in plate was done. 10µl from each conc. in 4wells i.e. 20 wells for one drug was added. Plate contained 5,000-10,000 cells/ml into each well of 96-well culture plate. The cells were incubated for 96 hr in CO2 incubator. After it cells are incubated with basal medium containing 0.5 mg/ml MTT in CO2 incubator at 37ºC for appropriate duration of time. The medium is aspirated, and the formazan product is solubilized with dimethyl sulfoxide (DMSO). Absorbance at 570 nm is measured for each well using a microplate reader on colorimeter. Analyse data of test with standard drug and plot graph.

Table No 1

S.NO	DILUTION	MEDIA	DRUG AMOUNT
1	100	1000µl	10 µg/ml from stock in 990 µ
2	10	90 µl	10 µg/ml from 1st in 90 µl
3	1	90 µl	10 µg/ml from 1st in 90 µl
4	.1	90 µl	10 µg/ml from 1st in 90 µl
5	0.01	90 µl	10 µg/ml from 1st in 90 µl

RESULT Table No 2

Cell Line	MCF-7			A-549			PA1		
Sample Code	1	2	3	1	2	3	1	2	3
IC50 (mg/ml)	3	15	45	20	40	45	10	30	40

Plant code- 1= *Withania Somnifera* , *2 = Capsicum annuum* ,*3 = Tamarindus indica*

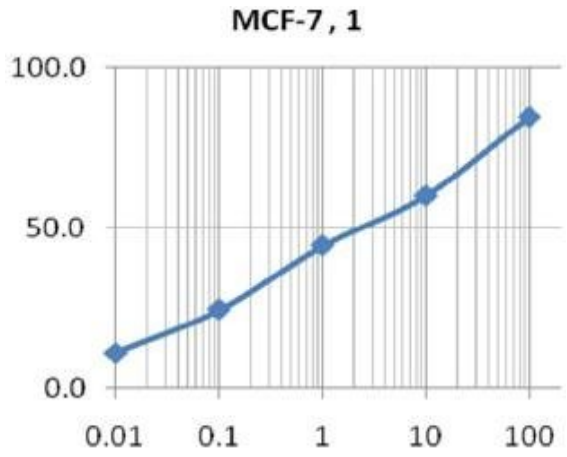

Fig- 1 MCF7 Cells were treated with hydro alcoholics extract of *Withania somnifera* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response Of MCF7 Cell to *Withania somnifera* For % Inhibition on Y-axis and Concentration on X-axis.

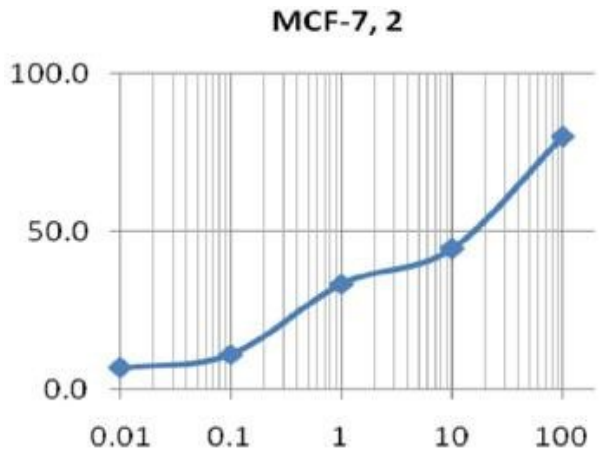

Fig- 2 MCF7 Cells were treated with hydro alcoholics of *Capsicum annuum* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response Of MCF7 Cell to *Capsicum annuum* For % Inhibition on Y-axis and Concentration on X-axis.

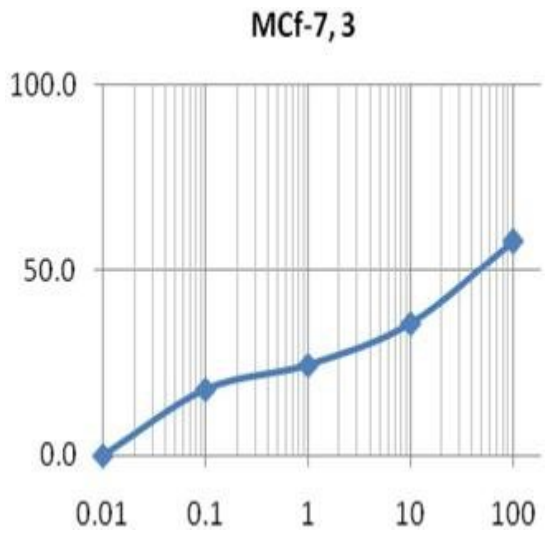

Fig- 3 MCF7 Cells were treated with hydro alcoholics of *Tamarindus indica* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response Of MCF7 Cell to *Tamarindus indica* For % Inhibition on Y-axis and Concentration on X-axis.

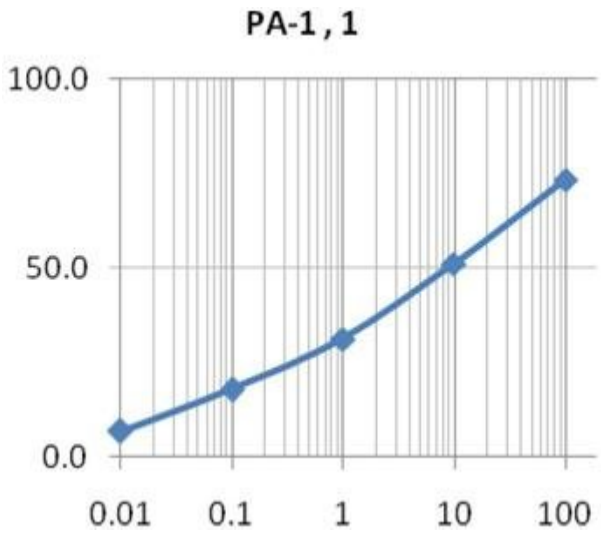

Fig- 4 PA1 Cells were treated hydro alcoholics extract of *Withania somnifera* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response Of PA1 Cell to *Withania somnifera* For % Inhibition on Y-axis and Concentration on X-axis.

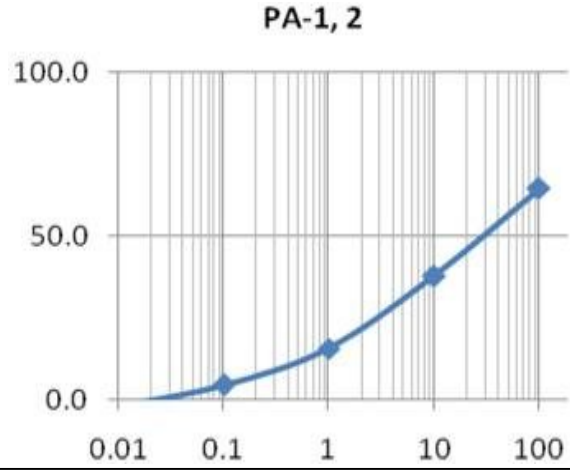

Fig- 5 PA1 Cells were treated with hydro alcoholics of *Capsicum annuum* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response Of PA1 Cell to *Capsicum annuum* For % Inhibition on Y-axis and Concentration on X-axis.

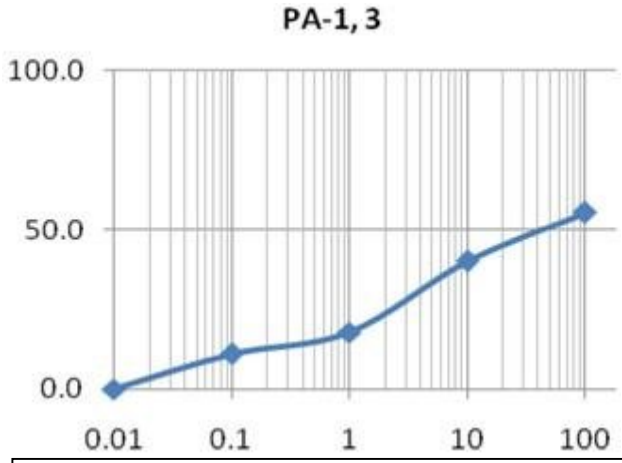

Fig- 6 PA1 Cells were treated with hydro alcoholics of *Tamarindus indica* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response Of PA1 Cell to *Tamarindus indica* For % Inhibition on Y-axis and Concentration on X-axis.

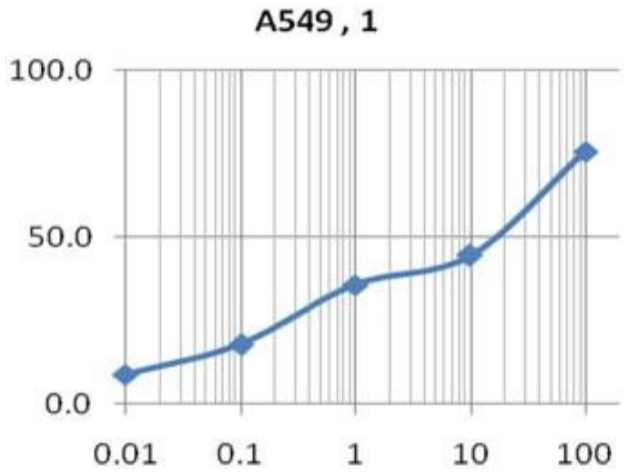

Fig- 7 A549 Cells were treated with hydro alcoholics extract of *Withania somnifera* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response Of A549 Cell to *Withania somnifera* For % Inhibition on Y-axis and Concentration on X-axis.

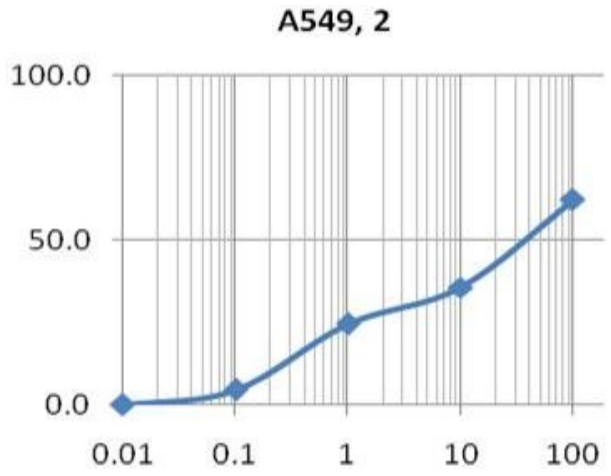

Fig- 8 A549 Cells were treated with hydro alcoholics of *Capsicum annuum* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. for 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response Of A549 Cell to *Capsicum annuum* For % Inhibition on Y-axis and Concentration on X-axis.

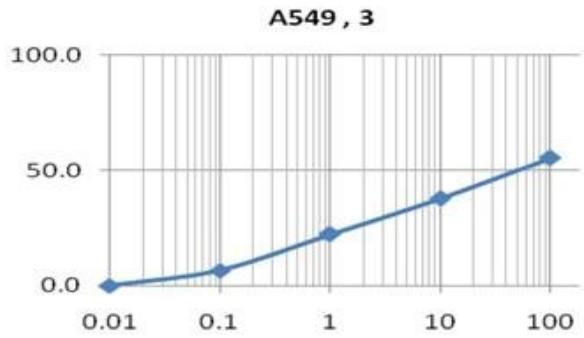

Fig- 9 A549 Cells were treated with hydro alcoholics of *Tamarindus indica* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 con. For 96 hr in a 96 well plate. Control cells received the solvent used to dissolve the extract. Cells were subjected to MTT within 1 hr- 24 hr. Response Of A549 Cell to *Tamarindus indica* For % Inhibition on Y-axis and Concentration on X-axis.

Conclude graph

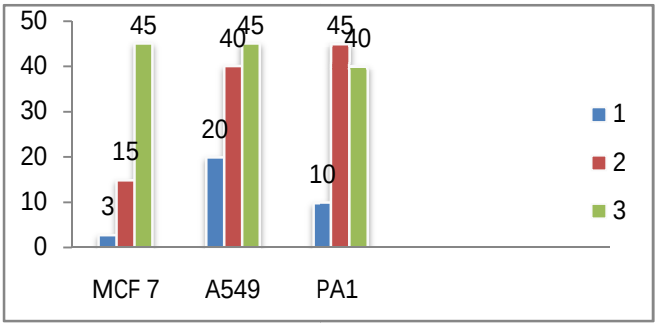

Fig- 10 This graph represents all three cell line and drug response on the basis of IC 50 value hears blue line (*Withania somnifera*) drug response higher than red (*Capsicum annum*) and green (*Tamarindus indica*) line drug.

DISCUSSION

During this study of three Indian medicinal plant extracts was investigated. The *invitro* cytotoxic potentiality was investigated as the ability of these three extracts to inhibit tumour cell line growth. With this investigation we had also focused on angiogenesis. The studied cell lines are MCF7, A549 and PA1. After exposure of cells to plant extract cell line were treated with MTT Dye which results into the live cells convert the MTT to purpled colour formazan crystals, which are soluble in Dimethyl sulphoxide (DMSO). After solubilisation of crystals then absorption is taken on spectrophotometer at 570 nm. With respect to readings the graphs were plotted for % inhibition on Y-axis and Conc. of drug on X-axis. The readings were directly converted into percentage. From above graph IC50 value of ***Capsicum annuum*** and ***Tamarindus indica*** for MCF7, A549,and PA1 is less as compare to ***Withania somnifera*** for MCF7, A549, PA1 Cell line.

CONCLUSION

In the present time herbal products are considered to be symbols of protection in comparison to the synthetic product that are regarded as unsafe to human life and environment. Although herbs had been priced for their medicinal importance, but now everyday phytochemical and pharmacological studies are conducted on different parts of these plants. More research can be done to investigate the unknown and unexplored potential of these plants. Further analysis of these plants (active compounds) can be carried out by way of making use of different analytical methods such as HPTLC, HPLC, FTIR, NMR and UV spectrophotometer analysis.

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