Research Article

Anticancer Activity of *Withania Somnifera (Leaves)* Flavonoids Compound

Rajeev Nema*[1], Sarita Khare, Parul Jain and Alka Pradhan
Sarojini Naidu Government Girls Post Graduate (Autonomous) College, Shivaji Nagar, Bhopal, India.
***Corresponding author's E-mail:** rrsht.nema@gmail.com

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ABSTRACT

In this research article make known on *Withania somnifera* (Ashwagandha) as Medicinal plants have therapeutic potential due to the presence of natural antioxidants functioning as reducing agents, free radical scavengers and quenchers of singlet oxygen. And this article also determines the use of *Withania Somnifera (leaves)* Polyphenolic Compound activity on MCF-7, A549 and PA-1 cancer cell line (breast, lung and ovary respectively). By providing a scientific basis the study can be made conventional to evaluate its constituents (natural product) to determine which of *Withania Somnifera (leaves)*, would facilitate further study as potential new anticancer agents or lead to new anticancer compounds. Hydro alcoholic (1:1) sample of *Withania Somnifera (leaves)* were prepared and tested for their cytotoxic activities against cancer cell lines (MCF7, A549and PA1) with standard Doxorubicin. The most essential reason of this study is to estimate cytotoxicity of certain important Indian medicinal plants with facilitate of MTT assay. Concentrations are set of each plant extract 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 *Withania Somnifera (leaves)* for 96 hr and then treated with MTT. For MTT absorbance in use at 570 nm. From IC_{50} values of MTT assay of *Withania Somnifera (leaves)* for MCF7, A549 and PA1 cancer cell lines, from this it may conclude that *Withania Somnifera (leaves)*shows efficient cytotoxicity on MCF-7 (10 ± 1 µg) than PA-1 (13 ± 1 µg) and A-459 (11± 1 µg) cancer cell line.

Keywords: Anticancer, Flavonoids, Medicinal Plants, *Withania somnifera*.

INTRODUCTION

Withania somnifera (Ashwagandha)

Scientific Classification:

Kingdom, Plantae; Order, Solanales; Family, Solanaceae; Genus, Withania; Species, *W. somnifera*.

Biological and Medicinal Property

The two main components of Ashwagandha Withaferin A and Withanolide E inhibit the growth of tumor showing a strong immune suppressive effect by stopping cancerous cells division. It is evident that foods rich in anti-oxidants play an important role in the prevention of cancer, cardiovascular and neurogenerative diseases. There has been a surge of research in its effect in animal models of atherosclerosis, hyperlipidemia, myocardial infarction, myocardial ischemia reperfusion injury, cerebral ischemia, cardiomyopathy, cardiac hypertrophy, cardiotoxicity and congestive heart failure. Many pharmacological studies have been conducted to investigate the properties of ashwagandha and to authenticate its use as a multi-purpose medicinal agent. Studies on *Withania somnifera* suggests that it reduces tumor cell proliferation and enhances the effectiveness of radiation therapy while potentially mitigating undesirable side effects[1]. The biological activities of *Withania somnifera* are anxiolytic-anti-depressive, antifungal[2], anti malarial[3], apoptotic[4], chondroprotective[5], cardioprotective[6], immunomodulator [7], neuroprotective[8], inhibition of COX-2 enzyme[9-10], promoter of learning and memory in Alzheimer's disease[11]. Sharada et.al. have studied toxicity of Withania somnifera root extract in rats and mice [12].

MATERIALS AND METHODS

Requirements

Alcohol 70%, 100% Alcohol, MEM media (Minimal Essential Media) (Eagle H 1959), Trypsin[13], MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide, a tetrazole)[14], Distilled Water, Dimethyl sulphoxide (DMSO)[15], etc. Laminar air flow, Autoclave, N2 liquid container, CO_2 incubator, Inverted microscope, Filtration assembly, Hemocytometer, Centrifuge machine, Micropipette, Soxhlet, Spectrophotometer.

Plant Material Collection

Withania Somnifera (leaves) plant ware collected from Bhopal during month of October. Than dried up under the shed dry for six week furthermore crush it.

Soxhlet Extraction: Hydroalcoholic (1:1)

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. *Withania Somnifera (leaves)* were extracted in Soxhlet Apparatus using Hydroalcoholic solvent (1:1).

Phytochemical Analysis

The hydroalcolic extract of *Withania Somnifera (leaves)* was tested for the presence of various phytoconstituents such as Carbohydrate, Starch, Protein, Aminoacids, Steroids, Flavonoids, Alkaloids, Tannins, Phenolic Compounds, oxalic acid and inorganic compounds. All

phytochemical tests were done as per the procedure given in the standard book (Practical Pharmacognocy by C.K.. Kokate). The FT-IR analysis of the *Withania Somnifera (leaves)* extract was done and the functional groups associated were determined.

Column chromatography

After phytochemical analysis bioactive compounds present in extract was separated out by column chromatography in a proper solvent system. Column chromatography was performed on a classic 20 cm long × 2 cm diameter glass column packed with 50 g Silica gel of 60-120 mesh size as stationary phase and crude drug were further subjected to column chromatography [CC] and eluted with specific solvent to obtain pure compounds. Silica gel for column chromatography was used as stationary phase. The flow rate used was 5 ml/min. Three and four elutes for each solvent were taken.

Spectrophotometric Determination of Total Flavonoid Content (TFC)

Total flavonoid contents were measured by Aluminum chloride colorimetric assay. Hydroalcoholic extracts that has been adjusted to come under the linearity range and different dilution of standard solution of Quercetin (10-100µg/ml) were added to 3ml of water. To the above mixture, 0.1ml of 5% $C_4H_4O_6KNa._4H_2O$ (Potassium Sodium L-(+) - Tartrate Tetrahdrate) was added. After 5 minutes, 0.1ml of 10% $AlCl_3$ was added and the total volume was made up to 3 ml with distilled water. It was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 430nm with a single beam spectrophotometer (Systronic) [16-17].

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 [18-19].

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 MCF-7 [20-21] (barest cancer), A-549 [22] (lung cancer) and PA-1 [23] (ovary cancer).

Assay Performed

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 is 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 5-10×10 3 cells/ml into *-each well of 96-well culture plate. The cells were incubated for 96 hr in CO_2 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 [20-24].

RESULTS AND DISCUSSION

Phytochemical Evaluation

The results of preliminary phytochemical evaluation are summarized in table 1.

Table 1: Phytochemical result list

Natural Product	Test Performed	Result
Carbohydrate	Molish Test	+Ve
Starch	Iodine	-Ve
Protein	Millions	+Ve
Amino Acid	Cysteine Test	+Ve
Steroid	Salkowski Test	+Ve
Flavonoids		+Ve
Alkaloid	Mayer's Test	+Ve
Tannic And Phenolic Compound	%5 $fecl_3$ Test	+Ve
Oxalic Acid		+Ve
Inorganic Acid	Sulphate Test	+Ve

Column chromatography

Column chromatography of *Withania Somnifera (leaves)* was performed on a classic 20 cm long × 2 cm diameter glass column packed with 50 g Silica gel of 60-120 mesh size as stationary phase and *Withania Somnifera (leaves)* crude drug were further subjected to column chromatography [CC] and eluted with specific solvent chloroform methanol water (1:2:1) to obtain pure compounds.

FT-IR Spectral Analysis

The FT-IR analysis of the samples was done and the functional groups associated were determined.The FT-IR spectrum of the sample was obtained effective peaks. the FT-IR spectrum of the *Withania Somnifera (leaves)* samples recorded the number of peaks lying between 3320.29 cm-1,2945.67 cm-1, 2834.64 cm- 1,1652.83 cm-1,1449.39cm-1,1417.14 cm-1,1113.62cm- 1,1016.45cm-1,755.15 cm-1,575.61 cm-1,546.14 cm- 1,534.78 cm-1, 510.12 cm-1 respectively. This finding helps in further research in the investigation of other medicinal plant with different solvent fraction for their antioxidant activity and it also useful to utilize of *Withania Somnifera (leaves)* plant as a source medicine.

Quantification

Current study revealed the flavonoid scontents of the leaves, of *Withania Somnifera (leaves).* (Quercetin standard plot: y =0.0966x, R2=0.9878)[25]. On the basis that calibration curve was plotted by preparing the Quercetin solutions at concentrations 12.5 mg/ml^{-1}. Total flavonoid content of the extracts was expressed as percentage of flavonoid in plant extract 4.78 equivalents per dry weight of sample and take notice of (Fig.3) y=0.002x+0.004, R2 =0.999.

MTT Assay Result

Cell Line	MCF-7	A-549	PA1
Sample Code	*Ws*	*Ws*	*Ws*
IC_{50} (mg/ml)	10±1	13±1	11±1

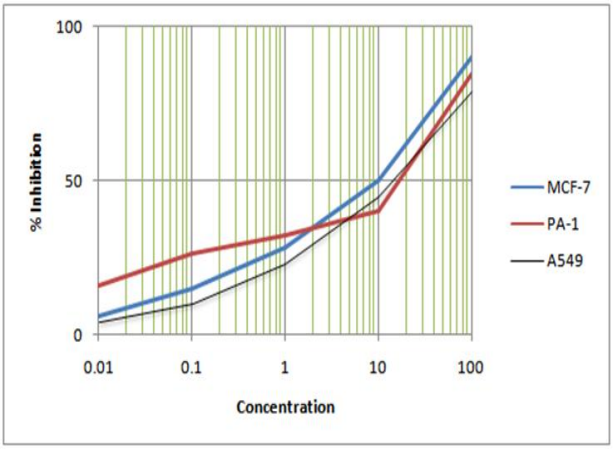

Figure 1: MCF-7,A549 and PA-1 Cells were treated with hydro alcoholics extract (Column pure compounds) of *Withania Somnifera (leaves)* dissolved in DMSO at 0.01, 0.1, 1, 10, 100 conc. Cells were subjected to MTT within 1 hr- 24 hr. Response of MCF7 Cell to *Withania Somnifera (leaves)* For % Inhibition on Y-axis and Concentration on X-axis.

DISCUSSION

Withania Somnifera (leaves) extract was investigated. The *invitro* cytotoxic potentiality was investigated as the ability of *Withania Somnifera (leaves)* 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 *Withania Somnifera (leaves)* 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 this it may conclude that *Withania Somnifera (leaves)*shows efficient cytotoxicity on MCF-7 (10 ± 1 µg) than PA-1(13 ± 1 µg) and A459 (11± 1 µg) cancer cell line where Standard drug was used for IC50 of Doxorubicin MCF-7 500nm, A549- 550nm, PA-1- 580nm.

CONCLUSION

More research can be done to investigate the unknown and unexplored potential of *Withania Somnifera (leaves).* Further analysis of *Withania Somnifera (leaves)* (active compounds) can be carried out by way of making use of different analytical and computer based methods such as HPTLC, HPLC, NMR and UV spectrophotometer and drug design analysis.

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