Anticancer Activity of Withania Somnifera (Leaves) Flavonoids Compound

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ABSTRACT

In this research article make known 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, A549 and 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 5-10x10 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 IC50 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, myopathy, 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. The biological activities of Withania somnifera are anxiolytic, anti-depressive, antifungal, anti malarial, apoptotic, chondroprotective, cardioprotective, immunomodulator, neuroprotective, inhibition of COX-2 enzyme, promoter of learning and memory in Alzheimer's disease. Sharada et.al. have studied toxicity of Withania somnifera root extract in rats and mice.

MATERIALS AND METHODS

Requirements

Alcohol 70%, 100% Alcohol, MEM media (Minimal Essential Media) (Eagle H 1959), Trypsin13, 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, CO2 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 hydroalcoholic 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) has been added to 3ml of water. To the above
mixture, 0.1ml of 5% C6H5O6KNaH2O (Potassium Sodium
L-(+) - Tartrate Tetrahydrate) was added. After 5 minutes,
0.1ml of 10% AlCl3 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) 18-17.

**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 (breast 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
CO2 incubator. After it cells are incubated with basal
medium containing 0.5 mg/ml MTT in CO2 incubator at
37°C 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 20-26.

**RESULTS AND DISCUSSION**

**Phytochemical Evaluation**

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

**Table 1: Phytochemical result list**

<table>
<thead>
<tr>
<th>Natural Product</th>
<th>Test Performed</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>Molish Test</td>
<td>+Ve</td>
</tr>
<tr>
<td>Starch</td>
<td>Iodine</td>
<td>-Ve</td>
</tr>
<tr>
<td>Protein</td>
<td>Millions</td>
<td>+Ve</td>
</tr>
<tr>
<td>Amino Acid</td>
<td>Cysteine Test</td>
<td>+Ve</td>
</tr>
<tr>
<td>Steroid</td>
<td>Salkowski Test</td>
<td>+Ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td>+Ve</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>Mayer’s Test</td>
<td>+Ve</td>
</tr>
<tr>
<td>Tannic And Phenolic Compound</td>
<td>%5 fecl3 Test</td>
<td>+Ve</td>
</tr>
<tr>
<td>Oxalic Acid</td>
<td></td>
<td>+Ve</td>
</tr>
<tr>
<td>Inorganic Acid</td>
<td>Sulphate Test</td>
<td>+Ve</td>
</tr>
</tbody>
</table>

**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,
1,1449.39cm-1,1417.14 cm-1,1113.62cm-1,1016.45cm-1,
1,755.15 cm-1,575.61 cm-1,549.12 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 contents of the leaves, of *Withania Somnifera* (leaves). (Quercetin standard plot: $y = 0.0966x$, $R^2 = 0.9878$)\(^{25}\). 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) $y = 0.002x + 0.004$, $R^2 = 0.999$.

**MTT Assay Result**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>MCF-7</th>
<th>A-549</th>
<th>PA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Code</td>
<td>Ws</td>
<td>Ws</td>
<td>Ws</td>
</tr>
<tr>
<td>IC(_{50}) (mg/ml)</td>
<td>10±1</td>
<td>13±1</td>
<td>11±1</td>
</tr>
</tbody>
</table>

**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 MCF7 (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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**REFERENCES**

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