essential oil from whole fruits (0.8%) were tested for antimicrobial potential. Table 1 shows the zone of inhibition in mm of methanol extracts of rind and fruits and essential oil of *Amomum subulatum* against microorganisms used.

Methanol extract of rind of *A. subulatum* showed remarkable antimicrobial activity against *E. coli*. When compared with ciprofloxacin, it was found equivalent in potency against *S. aureus*, whereas slight inferior against remaining bacterial stains. Methanol extract of fruits of *A. subulatum* was found to be effective against *S. aureus*. It was found equivalent to standard drug against *B. pumilus* and *P. aerugenosa*. Essential oil isolated from whole fruits showed good results against *B. pumilus*. It was found equivalent to the standard used against *S. epidermidis*, *P. aerugenosa* and *S. cerevisiae*. It was found that methanol extracts of fruit and rind as well as essential oil all possess antifungal activity but less than standard drug used.

It can be seen from the results obtained for MIC that rind extract of *A. subulatum* is having lower values as compared to fruit extract of *A. subulatum* in majority of microorganisms. Methanol extract of rind was showing exceptionally lower MIC in case of *S. aureus* (10 μg/disc).

The results obtained in the present study revealed that methanolic extracts of fruit and rind of *A. subulatum* possessed broad spectrum antimicrobial activity. The essential oil isolated from the whole fruits shows good antimicrobial activity against microorganisms used in the study.

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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\[1\]. Over 60% of anticancer drugs available in the market are of natural origin. Natural products are also the lead molecules for many of the drugs that are in use\[2\]. Therefore, the phytochemicals present in several herbal products and plants may have the potential to act as preventive or therapeutic agents against various human cancers\[3\]. The increased popularity of herbal remedies for cancer therapy perhaps can be attributed to the belief that herbal drugs provide benefit over that of allopathy medicines while being less toxic\[3\]. Since the conventional therapies have devastating side effects, there is a continuous need for search of new herbal cures of cancer\[4\].

**Withania somnifera** Dunal know as Ashwagandha belong Solanaceae family. It is extensively used in most of the Indian herbal pharmaceuticals and nutraceuticals. The current study, evaluate *in vitro* cytotoxicity in 50% ethanol extract of root, stem and leaves of *Withania somnifera* against five human cancer cell lines of four different tissues i.e. PC-3, DU-145 (prostate), HCT-15 (colon), A-549 (lung) and IMR-32 (neuroblastoma). Root, stem and leaves extracts showed cytotoxicity activity ranging 0-98% depending on the cell lines but maximum activity was found in 50% ethanol extract of leaves of *Withania somnifera*. Ethanol extract of leaves obtained from treatments T2, T3, T4 and T5 showed strong activity against PC-3 and HCT-15 with 80-98% growth inhibition, while the 50% ethanol extract of leaves from T1 treatment showed a minimum of 39% and T3 treatment showed a maximum of 98% growth inhibition against HCT-15. This investigation is the first report of the anticancer activity in various parts of *Withania somnifera* cultivated in fly ash amended soil.

Key words: Anticancer, cytotoxicity, fly ash, PC-3, HCT-15, prostrate, *Withania somnifera*

Ethanol GR Merck grade, RPMI-1640, fetal calf serum, trypsin, PBS, tryphan blue, penicillin, streptomycin, gentamycin, DMSO, sulphorhodamine, paclitaxel (taxol), 5-fluorouracil, were obtained from Sigma Chemical Co. USA and rest of the chemicals were of high purity and obtained locally. Tissue culture flasks and 96-Well cell culture plates were obtained from NUNC, Germany.

The *Withania somnifera* Dunal plant was cultivated in different concentration of fly ash amended soil at Advanced Materials and Processes Research Institute (CSIR), Bhopal, Madhya Pradesh, India. The experiments were designed to have five treatments, T1- control, T2- 5% fly ash, T3- 10% fly ash, T4-15% fly ash and T5- 20% fly ash. After completion of cropping root, stem and leaves of *Withania somnifera* were collected separately for each treatment and then air dried.

The dried roots stem and leaves samples of *Withania somnifera* Dunal were powdered separately and about 50 g of each powder was extracted with 50% ethyl alcohol in 1:10 w/v ratio for 24 h. by separating funnel. After many cycles, the desired compound was extracted in flask. This extract was concentrated using a Heidolph Rota Evaporator (Laboratra 4001, Germany) and concentrate was oven dried at 50°. The root, stem and leaves crude extract were utilized as test materials for *in vitro* anticancer activity testing.

Human cancer cell lines were procured from National Cancer Institute, Frederick, USA. Cells were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2 mM glutamine, pH 7.4, supplemented with 10% fetal calf serum, 100 μg/ml
streptomycin and 100 units/ml penicillin) in a carbon
dioxide incubator (37°, 5% CO₂, 90% RH). The cells
at sub confluent stage were harvested from the flask
by treatment with trypsin (0.05% in PBS (pH 7.4)
containing 0.02% EDTA). Cells with viability of more
than 98% as determined by trypan blue exclusion
were used for determination of cytotoxicity. The
cell suspension of 1×10⁶ cells/ml was prepared in
complete growth medium. Stock solutions (2×10⁻² M)
of compounds were prepared in DMSO. The stock
solutions were serially diluted with complete growth
medium containing 50 μg/ml of gentamycin to obtain
working test solutions of required concentrations.

In vitro cytotoxicity against four human cancer cell
lines was determined[13] using 96-well tissue culture
plates. One hundred microlitres of cell suspension
was added to each well of the 96-well tissue culture
plate. The cells were allowed to grow in a carbon
dioxide incubator (37°, 5% CO₂, 90% RH) for 24 h. Test materials in complete growth medium (100 μl)
were added after 24 h of incubation to the wells
containing cell suspension. The plates were further
incubated for 48 h. The cell growth was stopped
by gently layering trichloroacetic acid (50%, 50 μl)
on top of the medium in all the wells. The plates
were incubated at 4° for one hour to fix the cells
attached to the bottom of the wells. The liquid of all
the wells was gently pipetted out and discarded. The
plates were washed five times with distilled water
to remove trichloroacetic acid, growth medium low
molecular weight metabolites and serum proteins
and then air-dried. The plates were stained with
sulphorhodamine B dye (0.4 % in 1% acetic acid,
100 μl) for 30 min. The plates were washed five
times with 1% acetic acid and then air-dried[14]. The
adsorbed dye was dissolved in Tris-HCl Buffer (100 μl, 0.01M, pH 10.4) and plates were gently stirred for
10 min on a mechanical stirrer. The optical density
(OD) was recorded on ELISA reader at 540 nm. The
cell growth was determined by subtracting mean OD
value of respective blank from the mean OD value
of experimental set. Percent growth in presence of
test material was calculated considering the growth
in absence of any test material as 100% and in turn
percent growth inhibition in presence of test material
was statistically calculated.

Samples were evaluated against five cell lines
representing four different tissues. These were PC-3
and DU-145 from prostrate, HCT-15 from colon,
A-549 from lung and IMR-32 of neuroblastoma
origin. All root samples showed cytotoxicity ranging
between 0-46% depending on the cell lines but
maximum cytotoxicity activity was found in T3 and
T5, which showed more than 46% and 32% growth
inhibition in A-549 and DU-145 (Table 1). Stem
treatments showed cytotoxicity ranging between 4-64%.
Maximum cytotoxicity was found in T5 treatment
i.e. more than 71% growth inhibition against HCT-15
(colon). Stem extract of Withania somnifera grown in
fly ash-treated plot showed almost similar growth
inhibition against all cell lines, PC-3 and DU-145,
HCT-15, A-549 and IMR 032 (Table 2). The extract
of leaves showed cytotoxicity ranging between 27-98%,
which depended on the cell lines but maximum
cytotoxicity activity was found in T3 and T2 i.e.
more than 98% and 94% growth inhibition in HCT-15
(colon). Similarly the T4 and T5 treatments showed
greater growth inhibition against PC-3 and HCT-15,
while minimum cytotoxicity activity was found from
T1 treatment. Almost remarkable growth inhibition
was obtained from T3 treatment against all cell lines
studied (Table 3). The anticancer activity has been
previously reported in the Withania somnifera Dunal
against various human cell lines, but according to the

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration (μg/ml)</th>
<th>Prostate (PC-3)</th>
<th>Colon (HCT-15)</th>
<th>Lung (A-549)</th>
<th>Neuroblastoma (IMR-32)</th>
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</thead>
<tbody>
<tr>
<td>Root - T1</td>
<td>100</td>
<td>16</td>
<td>3</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Root - T2</td>
<td>100</td>
<td>12</td>
<td>9</td>
<td>4</td>
<td>22</td>
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<tr>
<td>Root - T3</td>
<td>100</td>
<td>40</td>
<td>12</td>
<td>13</td>
<td>46</td>
</tr>
<tr>
<td>Root - T4</td>
<td>100</td>
<td>20</td>
<td>14</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Root - T5</td>
<td>100</td>
<td>0</td>
<td>32</td>
<td>10</td>
<td>0</td>
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<tr>
<td>5- Fluorouracil</td>
<td>2 x 10⁻⁵ M</td>
<td>-</td>
<td>-</td>
<td>55</td>
<td>-</td>
</tr>
<tr>
<td>Adraimycin</td>
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</tr>
<tr>
<td>Paclitaxel</td>
<td>1 x 10⁻⁵ M</td>
<td>52</td>
<td>60</td>
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<td>-</td>
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</tbody>
</table>
literature anticancer activity in root, stem and leaves of *Withania somnifera* cultivated in fly ash amended soil has been reported probably for the first time.

In this study, growth inhibitory activity of root, stem and leaves of *Withania somnifera* using anticancer drugs paclitaxel, adriamycin and 5-fluorouracil (5-Fu) as positive controls was investigated against five cell lines representing four different tissues, PC-3, DU-145, HCT-15, A-549 and IMR-32. Although its root is well known for its importance in Ayurveda but reports on the medicinal activities of stem and leaves are very few. Thus this study revealed the enormous medicinal potential of stem and leaves of this plant. This study gives support that *Withania somnifera* 50% ethanol extract were highly cytotoxic to the human cell lines studied.

Earlier studies indicated that only the root extract of *Withania somnifera* as a potential source of new molecules that can curtail cancer growth\[^{15}\]. *Withania somnifera* leaves have also been shown to inhibit the growth of human cancer cell lines comparable to that produced by adriamycin. The leaf extract produced antiproliferative activity on NCI-H460 (lung), HCT-116 (colon), SF-268 (central nervous system) and MCF-7 (breast) human tumor cell lines. The inhibitory concentrations obtained was 25.1±0.91 against colon cell line HCT-116\[^{11}\], but in this study leaf extracts from T2, T3, T4 and T5 treatments of *Withania somnifera* cultivated in fly ash containing soil had shown more than 80% inhibition against PC-3 and HCT-15 cell lines. Further more this study has reported growth inhibitory importance in *Withania somnifera* against various human cancer cell lines i.e. PC-3, DU-145, HCT-15, A-549 and IMR-32. Hence, this study has revealed remarkable anticancer potential in the leaves of *Withania somnifera*.

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Eight novel 1-(substituted acetyl)-4-(10-bromo-8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidene)piperidines were designed by incorporating zinc binding groups to enhance activity. The designed molecules were synthesized and were evaluated for antitumor activity in vitro in five cell lines and for farnesyl protein transferase inhibition. Test compounds (6a-h) exhibited antitumor activity in most of the cell lines but were less potent than adriamycin. Compound 6e was most active with IC50 values of <15 µM in two cell lines tested. Test compounds also exhibited potent FPT inhibitory activity and 6c was most potent with IC50 value of <30 µM.

Key words: Anticancer agents, benzocycloheptapyridines, farnesyl protein transferase inhibitors

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