Anti-cancer Activity of Leaf Extract Preparation from *Ipomoea sepiaria* against PC-3 Cell Line

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Received: 01 June 2017/Revised: 25 July 2017/Accepted: 23 August 2017

**ABSTRACT** - Researches on PC-3 human prostate cancer cell lines control are needed in the present decades. The anticancer activity of aqueous extract of *Ipomoea sepiaria* was investigated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay using PC-3 cell line. The present experimentation was showed that aqueous extract of *Ipomoea sepiaria*, when subjected to different concentrations on PC-3 cells showed IC50 cell inhibition at about 5µM for 48 hours and about 2µM for 72 hours. PTEN interacts with other expression proteins like Jun proto-oncogene, V-akt murine thymoma viral oncogene homolog 1 and 2, Tumor protein p53, Phosphatidylinositol-4,5-bisphosphate 3-kinase, etc. The present experiment shown that the leaf extract may involve in protein suppressor mechanism for PTEN in controlling of prostate cancer.

**Key-words** - PC-3, *Ipomoea sepiaria*, Anticancer activity, MTT assay, Prostate cancer

**INTRODUCTION**

One of the source plants of the classical herb Lakshmana is *Ipomoea sepiaria* Koenig Ex. Roxb belongs to the family Convolvulaceae. *Ipomoea sepiaria* (I. sepiaria), a perennial climber is an important ethanomedicinal plant having phyto-constituents like alkaloids, carbohydrates, flavonoids, glycosides, saponin, tannin and phenolic compounds. PC3 (PC-3) is a human prostate cancer cell lines that are highly used in investigating the biochemical changes prostatic cancer cells. From the last few years, Phytotherapy (herbal therapies) usage for prostate cancer has been increasing dramatically. Several herbs like *Chrysanthemum morifolium* [3], *Ganoderma lucidum* (a root fungus) [4], *Glycyrrhiza glabra* (Spanish liquorice) [5], *Scutellaria baicalensis* [6], *Panax pseudoginseng* [7], *Dendranthema morifolium* [8], *Rabdosia rubescens* [9], and *Isatis indigotica* [10] are tested effective in the treatment of PC-3 cell lines. There were no reports for using *Ipomoea sepiaria* as a medicinal plant that was used against PC-3 cell line for treating prostatic cancer.

A candidate tumor suppressor gene, PTEN (putative protein tyrosine phosphatase) gene is considered as the responsibility of causing prostate cancer [11]. Research about risks and benefits of human prostate cancer screening and treatments are needed in the present decades [12]. Protein interaction studies provide better clues in understanding control mechanisms involved in phytotherapy.

**MATERIALS AND METHODS**

**Collection of plant material**

Fresh leaves of *Ipomoea sepiaria* were collected from surrounding areas of Visakhapatnam, India during March 2017 and the work is conducted at Department of Biotechnology, GITAM University, Visakhapatnam, India. The dust particles were removed by washing leaves of *Ipomoea sepiaria* with double distilled water. The leaves were shade dried and then grounded to powder using mortar and pestle. The obtained powdered samples were then stored in an airtight closed bottle and were used for further experiments.

**Preparation of plant extract of *Ipomoea sepiaria***

About 20gms of the powder of *Ipomoea sepiaria* plant leaves were taken in 250 ml Erlenmeyer flask. The material was boiled with 100 ml of double distilled water, filtered with Whatman Filter paper No. 1 after cooling and was stored at 4°C for further experimentation.
Anticancer Activity of *Ipomoea sepiaria* against PC-3 cell lines using MTT Assay

To investigated the *in vitro* inhibitory effects of the aqueous extract from leaves of *Ipomoea sepiaria*, PC-3 procured from NCCS, Pune, India and sensitivity of PC-3 to *Ipomoea sepiaria* were determined by the MTT colorimetric assay. About 5000 to 10000 cells approximately in 100 µl MEM media (MEM199, Sigma, India) per well was seeded in a 96 well plate and incubated at 37°C, 5% CO₂ for 72 hours. The cells were exposed to leaf extract *Ipomoea sepiaria* at 6 concentrations 0µM, 1µM, 2µM, 5µM, 10µM and 20µM. The cells were then treated with, 20µl of freshly prepared MTT reagent (5mg/ml in PBS) was added and then DMSO (200 µl) was added to each well to dissolve the formazan crystals. The absorbance (OD) of the culture plate was read at a wavelength of 492 nm on an ELISA reader, AnthosBiochrom 2020 ELISA Reader. The percentage of residual cell viability were determined based on the absorbance (OD) obtained from ELISA Reader.

**RESULTS AND DISCUSSION**

Anticancer activity of *Ipomoea sepiaria* against PC-3 cell line MTT assay

The anticancer activity of aqueous extract was investigated by MTT assay. The present experimentation showed that aqueous extraction of *Ipomoea sepiaria* when subjected to different concentrations on PC-3 cells showed IC50 cell inhibition of at about 5µM for 48 hours and about 2µM for 72 hours (Table 1, Fig 1).

### Table 1: Evaluation cytotoxic property of the extract on PC-3 cells by MTT assay

<table>
<thead>
<tr>
<th>Time</th>
<th>Concentration</th>
<th>Raw data A1</th>
<th>Raw data A2</th>
<th>Raw data A3</th>
<th>Average</th>
<th>SD</th>
<th>% Cell survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>48h</td>
<td>Control</td>
<td>1</td>
<td>1</td>
<td>1.07809</td>
<td>1.03</td>
<td>0.05</td>
<td>100</td>
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<tr>
<td></td>
<td>1µM</td>
<td>0.9</td>
<td>1</td>
<td>1</td>
<td>0.97</td>
<td>0.06</td>
<td>89.5</td>
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<tr>
<td></td>
<td>2µM</td>
<td>0.6</td>
<td>0.6</td>
<td>0.7</td>
<td>0.63</td>
<td>0.06</td>
<td>58.6</td>
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<tr>
<td></td>
<td>5µM</td>
<td>0.59698</td>
<td>0.6005</td>
<td>0.65</td>
<td>0.62</td>
<td>0.03</td>
<td>57</td>
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<tr>
<td></td>
<td>10µM</td>
<td>0.5</td>
<td>0.4</td>
<td>0.5</td>
<td>0.47</td>
<td>0.06</td>
<td>43.2</td>
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<tr>
<td></td>
<td>20µM</td>
<td>0.2</td>
<td>0.4</td>
<td>0.36625</td>
<td>0.32</td>
<td>0.11</td>
<td>29.8</td>
</tr>
<tr>
<td>72h</td>
<td>Control</td>
<td>1</td>
<td>0.9486</td>
<td>1.00259</td>
<td>0.98</td>
<td>0.03</td>
<td>100</td>
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<tr>
<td></td>
<td>1µM</td>
<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
<td>0.83</td>
<td>0.06</td>
<td>85.0</td>
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<tr>
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<td>0.5</td>
<td>0.50026</td>
<td>0.53</td>
<td>0.06</td>
<td>54.4</td>
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<tr>
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<td>0.4</td>
<td>0.4</td>
<td>0.43</td>
<td>0.06</td>
<td>44.2</td>
</tr>
<tr>
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<td>10µM</td>
<td>0.3</td>
<td>0.4</td>
<td>0.2</td>
<td>0.30</td>
<td>0.10</td>
<td>30.6</td>
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<tr>
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<td>0.2</td>
<td>0.3</td>
<td>0.23</td>
<td>0.06</td>
<td>23.8</td>
</tr>
</tbody>
</table>

Control = mononuclear cells isolated from buffy coats and PC-3 cells

String database (https://string-db.org/) is applied for the investigation of protein interaction (PTEN) in human.
Fig. 2 was shown that PTEN interacts with other expression proteins like Jun proto-oncogene, V-akt murine thymoma viral oncogene homolog 1 and 2, Tumor protein p53, Phosphatidylinositol-4, 5-bisphosphate 3-kinase etc. The leaf extract may show protein suppressor mechanism for PTEN in controlling of prostate cancer.

Prostate cancer is a leading cause of cancer death, in human that was related to the metastatic disease caused due to mutations and different gene expressions [13]. Inactivation of PTEN/MMAC1 can cure human prostate cancer through loss of expression mechanism [14].

PC3 (PC-3) are prostate cancer cell lines that are used extensively in prostate cancer research [15]. Many successful drugs from natural products are acting as an important source for the isolation and activities as anti-cancer lead molecules [16]. The molecules may lead to the control and cure of diseases involved in ageing diseases.

Uncontrolled growth due to external factors and internal factors within the biological systems that finally results in death of cells or stopping functionality of components within systems is termed as cancer. Plants have many phytoconstituents like alkaloids, flavonoids, coumarins, polyphenols etc., possesses good antitumor properties [17-18].

In the present decades, in silico docking approaches are providing exploring information through the studies of physicochemical characteristics like angiogenesis, diabetes, growth and repair of cancerous cells. Alkaloids from biological sources have good inhibitory effect on cancer cell proliferation [19-20].

MTT assay is based on the enzymatic reduction of the tetrazolium salt MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazoliumbromide] for the quantitation measurements of growth modulating effects on the cultured prostate cancer cell lines. The MTT test provides easy and high degree of accuracy measures, hence the test is suitable for large scale purpose of chemosensitivity testing [21].

Table 2 was shown the usage of medicinal plants acting as anti-cancer agents hat were analyzed using MTT assay. The experimentation of Ipomoea sepiaria against MCF-7 cell lines were previously experimented by Sudhakar and Kaladhar, 2017 [28]. Experimentation on anticancer activity of aqueous leaf extract from Ipomoea sepiaria by MTT assay using PC-3 cell line has been not reported till date.

**CONCLUSIONS**

The present study has shown that an aqueous extract of *Ipomoea sepiaria* had considerable anti-cancer activity against prostate cancer cell lines. In silico protein interaction studies may proposed the activation of *Ipomoea sepiaria* may control PTEN molecule that was involved in prostate cancer. These results have shown us a path to conduct in vivo experiments to evaluate the extract of Indian *Ipomoea sepiaria*.
ACKNOWLEDGMENT
The authors would like to thank JNTUH, GITAM University and Bilaspur University for providing necessary facilities, financial support and technical assistance in bringing out the research work.

REFERENCES


**How to cite this article:**

**Source of Financial Support:** Nil, **Conflict of interest:** Nil