

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

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ABSTRACT- Researches on PC-3 human prostate cancer cell lines control are needed in the present decades. The anticancer activity of the aqueous extract of *Ipomoea sepiaria* was investigated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay using the PC-3 cell line. The present experimentation was showed that aqueous extract of *I. sepiaria*, when subjected to different concentrations of PC-3 cells showed IC50 cell inhibition at about 5 μ M for 48 hours and about 2 μ M 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. *I. 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 [1,2]. 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 from using *I. 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 the 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 *I. sepiaria* were collected from surrounding areas of Visakhapatnam, India during March 2017 and the work is conducted at the Department of Biotechnology, GITAM University, Visakhapatnam, India. The dust particles were removed by washing the leaves of *I. 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 *I. sepiaria*

About 20 gm of the powder of *I. 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.

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Anticancer Activity of *I. sepiaria* against PC-3 cell lines using MTT Assay

We investigated the *in vitro* inhibitory effects of the aqueous extract from leaves of *I. 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 *I. sepiaria* at 6 concentrations 0 µM, 1 µM, 2 µM, 5 µM, 10 µM and 20 µM. The cells

were then treated with 20 µl of freshly prepared MTT reagent (5 mg/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 was determined based on the absorbance (OD) obtained by ELISA Reader.

String

String database (<https://string-db.org/>) is applied for the investigation of protein interaction (PTEN) in human.

RESULTS AND DISCUSSION

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

The anticancer activity of the aqueous extract was investigated by MTT assay. The present experimentation

showed that aqueous-extraction of *I. sepiaria* when subjected to different concentrations of PC-3 cells were shown IC₅₀ cell inhibition of at about 5 µM for 48 hours and about 2 µM for 72 hours (Table 1, Fig. 1).

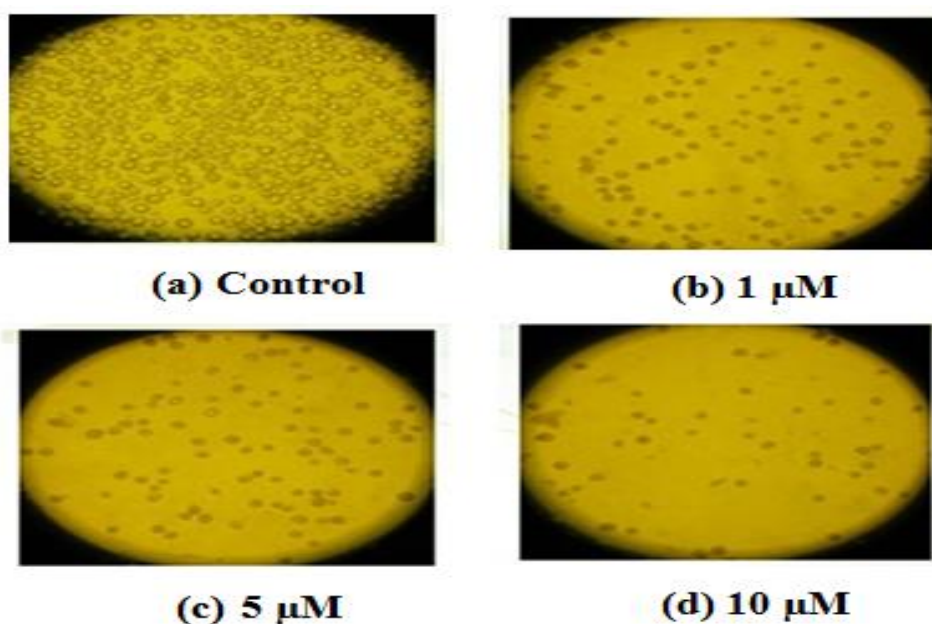


Fig. 1: Cultures of PC-3 cells for 48 hours *I. sepiaria* extract treatment

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

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

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

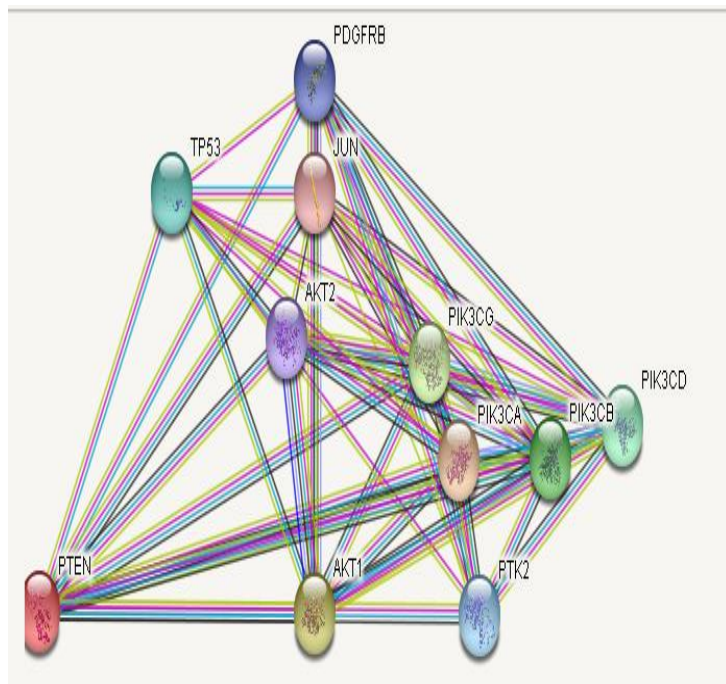


Fig. 2: String database for protein interaction studies for PTEN

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 humans 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 the 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 a better 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 the large scale purpose of chemosensitivity testing [21].

Table 2: Previous studies conducted by MTT assay from Medicinal Plants

Medicinal Plant	Cell line	Reference
<i>Solanum nigrum</i>	HeLa and Vero cell line	Patel <i>et al.</i> [22]
<i>Garcinia mangostana</i>	SKBR3 human breast adenocarcinoma cell line	Moongkarndi <i>et al.</i> [23]
<i>W. somnifera</i>	NCI-H460 (Lung), HCT-116 (Colon), SF-268 (Central Nervous System) and MCF-7 (Breast) human tumor cell lines	Jayaprakasam <i>et al.</i> [24]
<i>Crocus sativus</i> L	HepG2 and HeLa cell lines	Tavakkol-Afshari <i>et al.</i> [25]
<i>Phaleria macrocarpa</i> (Scheff.) Boerl	esophageal cancer cells (TE-2)	Faried <i>et al.</i> [26]
<i>Piper longum</i>	Hep-2	Jacob <i>et al.</i> [27]

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

CONCLUSIONS

The present study has shown that an aqueous extract of *I. sepiaria* had considerable anti-cancer activity against prostate cancer cell lines. *In silico* protein interaction studies may propose the activation of phyto-compounds from *I. 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 *I. sepiaria*.

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