Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Anti-Cancer Activity of *Smilax wightii* A. DC. (Smilacaceae), an Endemic Medicinal Plant on Prostrate (Pc-3) and Cervical Cancer (Hela) Cell Lines.

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ABSTRACT

The present study was carried out to evaluate the *in-vitro* cytotoxic activity of methanolic leaf extract of *Smilax wightii* against human prostate cancer cell line (PC-3) and cervical cancer (HeLa) cell lines by MTT assay. The leaf extract decreased cell viability, inhibited cell proliferation, and induced cell death in a dose dependent manner. The methanolic extract of *S. wightii* exhibited significant cytotoxic activity against human prostate cancer (PC-3) and cervical cancer (HeLa) cell lines and with IC_{50} values of 38.22µg/mL and 27.95µg/mL respectively.

KEYWORDS: *Smilax wightii*, MTT-assay, human prostate cancer and cervical cancer cell lines.

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INTRODUCTION

Cancer is a hyper proliferative disorder that involves transformation, dysregulation of apoptosis, proliferation, invasion, angiogenesis and metastasis. Drugs used to treat most cancers are those that can block cell signalling, including growth factor signalling. Numerous reports have suggested that Ayurvedic plants and their components mediate their effects by modulating several of these recently identified therapeutic targets.[1] In Western medicine, only a limited number of plant products are being used to treat cancer. However, some of the widely used anticancer drugs, such as taxol and vinca alkaloids, are obtained from plants.[2] *Smilax* is a genus which has more than 300 species, found on temperate , tropic and subtropic zones in worldwide.[3] *Smilax wightii* A.DC. (Smilacaceae) is a large woody climber and this genus has many pharmacological properties as used in curing the diseases like cancer, diabetes mellitus, skin ailments including wounds, inflammations, boils and ulcers.[4]

Of the 121 medicines of the drugs in utilize for cancer treatment, 90 are derived from plants. Almost 74% of these, including taxol, were discovered by investigating a folkore claim.[5,6] These phytochemicals are commonly called chemotherapeutic or chemopreventive agents. Phytochemicals may fight disease through suppression of the inflammatory response. Dysregulated inflammation contributes to many diseases, including cancer.[7] It is likely that dietary constituents such as garlic, ginger, soya, curcumin, onion, tomatoes, cruciferous vegetables, chillies and green tea, play an important role in protection from these cancers. These dietary agents are believed to suppress the transformative, hyperproliferative and inflammatory process that initiate carcinogenesis. Because these chemoprotective agents are derived from natural sources, they are considered pharmacologically safe.[1]

MATERIALS AND METHODS

Collection Of The Plant Material

Smilax wightii whole plant was collected from Kodanadu, The Nilgiri Hills, The Western Ghats, Southern India. The plant was identified and authenticated by a plant taxonomist, Dr. M. Murugesan, SACON, Coimbatore, India.

Preparation of Extracts

The plant leaves were dried in shade and then powdered using dry grinder. 100 g of dried plant leaf powder was extracted in 500ml of methanol in an orbital shaker for 72 hrs. Repeated extraction was done with the same solvent till clear colourless solvent was obtained. The extract was concentrated to dryness and at least 20% of extractive was obtained. The filtrate was stored at 2-8°C

in an air tight container for further experimentation. This methanolic extract was dissolved in DMSO and made in to stock solution.

Human Cell Lines

The human prostate cancer (PC-3) and human cervical cancer (HeLa) cell lines were obtained from the National centre for cell sciences, Pune (NCCS), India. PC-3 and HeLa cells were cultured in Minimum Essential Media (MEM) with Earle Salt without glutamine medium supplemented with 10% heated fetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/ml penicillin, 50 µg/ml streptomycin, 1% non-essential amino acid and maintained at 37ºC in a 5% CO_2 atmosphere with 95% humidity.[8] According to their growth profiles, the optimal plating densities of PC-3 and HeLa cancer cell lines were determined by $3x10^3$ cells/well to ensure exponential growth throughout the experimental period and to ensure a linear relationship between absorbance at 570 nm and cell number which was analyzed by MTT assay

MTT [(3,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide)] Assay

The MTT assay was performed according to a slight modification of the procedure reported by.[9] Cells were cultured in minimum essential medium (MEM) supplemented with glutamine (0.6g/L), gentamicin (25mg/ml), 10% fetal calf serum at 37ºC and in humidified 5% CO_2. For experiments, cells were plated in 96-well plate (105 cells/ well for adherent cells or 0.3x106 cells/ well for suspended cells in 100 µL of medium). After 24 h, the extracts (0.01, 0.1, 1, 10 and 100 µg/ml) dissolved in DMSO (1%) was added to each well and incubated for 96 h. The control groups received the same amount of DMSO. Doxorubicin (0.01, 0.1, 1, 10, 100µg/ml) was used as positive control. Growths of tumoral cells were quantified by the ability of living cells to reduce the yellow dye MTT to a blue formazan product. At the end of 96h incubation, the medium in each well was replaced by fresh medium containing 0.5mg/ml of MTT. Four hour later, the formazan product of MTT reduction was dissolved in DMSO and absorbance was measured at 570 nm. Drug effect was quantified as the percentage of control absorbance of reduced dye at 570 nm. Percentage inhibitions [100 - (absorbance of test wells/absorbance of control wells) x100] were calculated and plotted against the concentrations used to calculate the IC_{50}.

Statistical Analysis

The data is expressed as mean ± SEM and subjected to one way ANOVA and the level of significance was set at $p < 0.05$. The IC_{50} (Inhibitory concentration) value obtained from MTT assay was calculated using Microsoft excel software.

RESULTS AND DISCUSSION

In vitro cytotoxic activity of *S.wightii* plant leaf extract was evaluated against human prostate cancer (PC-3) and cervical cancer (HeLa) cell lines. The methanolic leaf extract of *S. wightii* was screened for its cytotoxic activity against these two cell lines at various concentrations in order to determine the IC_{50} value (50% growth inhibition) by MTT assay. The dose dependent responses of the human prostate cancer (PC-3) and cervical cancer (HeLa) cell lines are shown in Table-1.The methanolic leaf extract of *S. wightii* exhibited significant cytotoxic activity against human prostate cancer (PC-3) and cervical cancer (HeLa) cell lines with IC_{50} values of 38.22 µg/mL and 27.95 µ/mL respectively.

Table: 1 *In Vitro* Cytotoxic Effect Of Methanolic Leaf Extract Of *S. wightii* On Human Prostrate And Cervical Cancer Cell Lines.

S. No	Concentration (µg/mL)	Methanolic leaf extract of *S. wightii*	
		Prostate cancer (PC-3)	Cervical cancer (HeLa)
1	250	14.44±1.32	24.47±1.29
2	125	17.44±0.34	28.22±0.34
3	62.5	35.58±0.68	36.49±0.73
4	31.25	44.07±0.38	44.39±0.61
5	15.62	55.55±0.68	55.02±0.95
6	7.81	56.70±0.71	59.51±0.38
7	3.90	66.17±0.92	63.69±1.22
8	Cell control	100	100
IC_{50}		**38.22µg/mL**	**27.95µg/mL**

The cells treated with *S. wightii* extract were examined for morphological changes using an inverted microscope and compared with the cells of the control group (Fig1. and 2.).

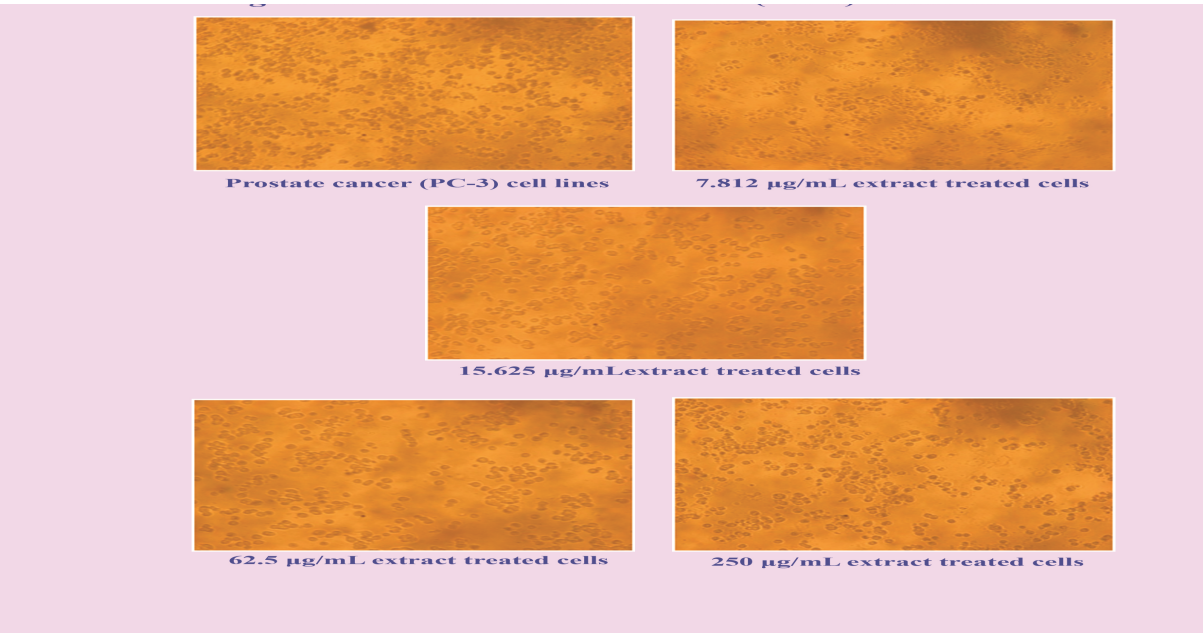

Fig1. *In Vitro* Cytotoxic Effect Of Methanolic Leaf Extract Of *S. wightii* On Human Prostrate Cancer (PC-3) Cell Lines.

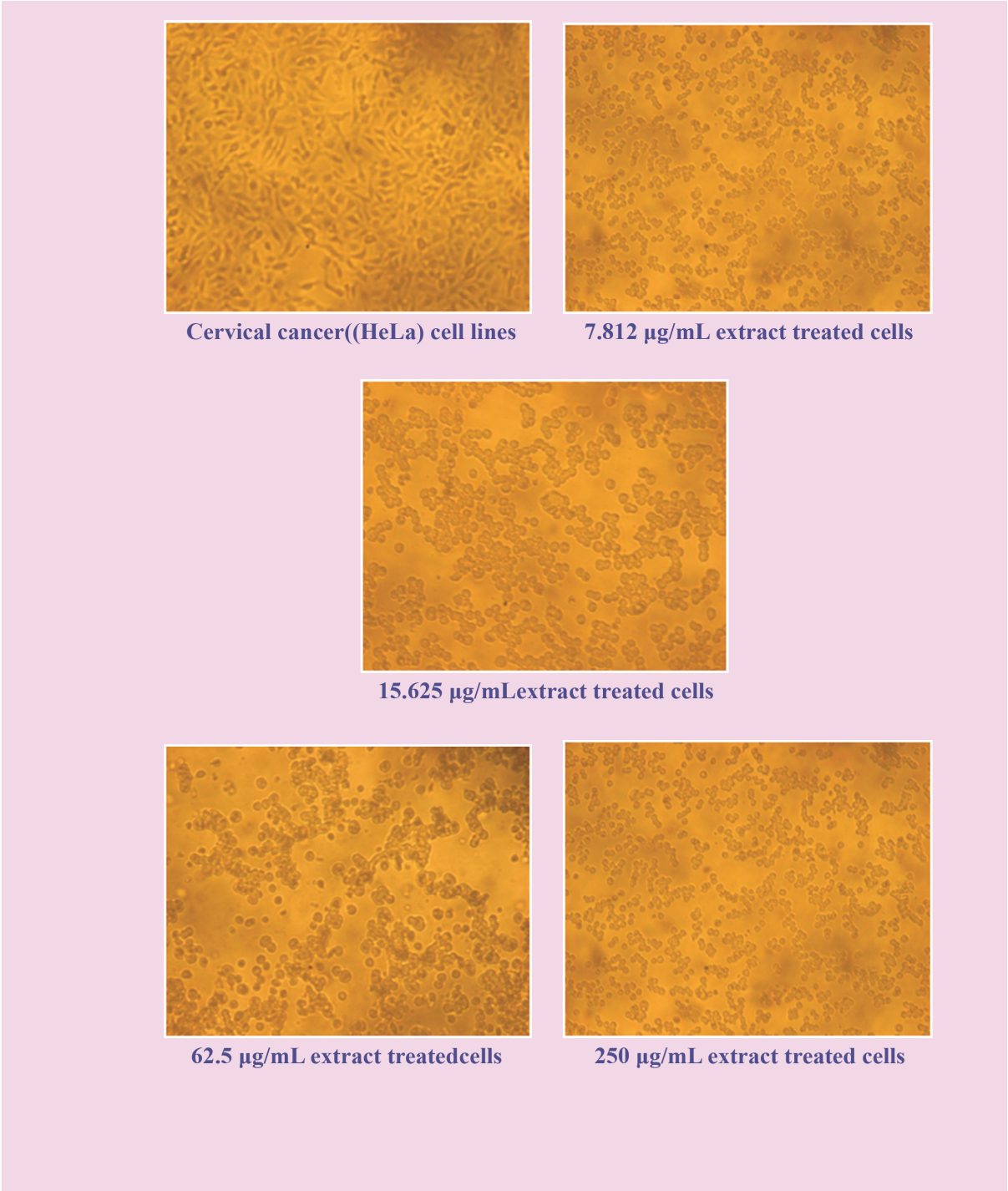

Fig2. *In Vitro* Cytotoxic Effect Of Methanolic Leaf Extract Of *S. wightii* On Human Cervical Cancer (Hela) Cell Lines.

The methanolic leaf extract of *S. wightii* extract showed cytotoxic activity on prostrate and cervical cancer cell lines, where the differences between the control and the treatment groups were significantly different ($P<0.05$). The extract of *S. wightii* suppressed cell proliferation and the difference in mean was significant compared to the control ($P>0.05$) in Fig 1and 2.

Medicinal plants constitute a common alternative for cancer prevention and treatment in many countries around the world.[10] Approximately, 60% of the anticancer drugs currently used have been isolated from natural products from the plants. At this time, more than 3000 plants worldwide have been reported to possess anticancer properties.[11] The parts of plants viz., inflorescence (*Artemisia vulgaris*), seeds (*Cichorium intybus*), rhizome (*Smilax glabra*), berries (*Solanum nigrum*) and whole plant (*Swertia chirayata*) have been commonly used in traditional Indian medicine for the treatment of various human ailments for many years.[12] Extracts of these medicinal plants are believed to contain a wide array of polyphenolic compounds which might possess cancer preventive and/or therapeutic properties.[11]

Some of the species in Smilacaceae family such as *S. zeylanica* and *S. aspera* are used in treating stomach, skin, breast, cervix and throat cancers.[13] The Kanikkar tribes, inhabitants of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu, India use the paste prepared from tubers of *S. wightii* with water to treat cancer.[14] The cytotoxic activity of crude ethanol extract showed a potent inhibition with an ED_{50} value at 31µg/ml. The pure constituents from *S. venosa* could be active as new drug for ovarian cancer.

The present study also determined whether the extracts of *S. wightii* exerted an inhibitory effect on cancer cell proliferation and cause cell death. The results suggested that the methanol extract of *S. wightii* possess the strongest cytotoxic effects on prostate (PC-3) and cervical (HeLa) human cancer cells. The preliminary phytochemical study showed the presence of alkaloids, flavonoids, terpenoids, saponins, phenol, tannins and glycosides which are known for their anticancer properties. The phytochemical constituents of *Artemisia* sp. viz. artemisinin was approved for use in humans as an anti-malarial drug.[15,16] Hence, unravelling the anticancer properties artemisinin and the underlying mechanisms will be critical to determine which cancer phenotype can best be treated with this phytochemical and for testing and pharmacological characterization in humans. Another study has reported that constituents isolated from *Artemisia vestita* exhibited marked anticancer property in inhibiting human pancreatic cancer cell proliferation.[17] The present cytotoxicity study was carried in the methanolic leaf extract of *S. wightii*. These extracts were screened for their cytotoxicity against prostate (PC-3) and cervical (HeLa) cancer cell lines at different concentrations to determine the IC_{50} values (50% growth inhibition) by MTT assay.

CONCLUSION

The results of the present study show that the methanolic extract of *S.wightii* has exhibited a potential cytotoxic effect on prostate and cervical cancer cell lines.

REFERENCES

1. Bharat BA, Haruyo I, Prachi G, Priya W et al. "*From traditional Ayurvedic medicine to modern medicine: identification of therapeutic targets for suppression of inflammation and cancer*", Expert Opinion on Therapeutic. Targets 2006; 10 (1): 87-118.
2. Newman DJ, Cragg GM and Snade KM, "*Natural products as sources of new drugs over the period 1981-2002*", Journal of Natural Products 2017; 66 :1022-1037.
3. Fnaec, "*Flora of North America editorial committee*", Flora of North America , North of Mexico 2002; 6 :14–46.
4. Damayanthi DA , Mab A, Ahk A et al."*Effects of Smilax myosotiflora on testicular 11a-hydroxysteroid dehydrogenase oxidative activity and plasma hormone levels in rats*", Biomedical Research 2011; 22 :188–193.
5. Craig WJ, "*Phytochemicals: guardians of our health*", Journal of the American. Dietetic Association 1997; 97: S199 - S204.
6. Craig WJ, "*Health-promoting properties of common herbs*", The American Journal of Clinical Nutrition 1999; 70: 491S-499S.
7. Coussens LM and Werb Z. "*Infammation and cancer*", Nature 2002; 420: 860-867.
8. Itharat A, Houghton PJ, Eno-Ammguaye E , Burke PJ et al. " *In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer*", Journal of Ethnopharmacology2004; 90: 33- 38.
9. Mosmann T, "*Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays*", Journal of Immunological Methods 1983; 65 (1-2): 55-63.
10. Desai AG , Qazi GN, Ganju RK et al. "*Medicinal plants and cancer chemoprevention*",Current Drug Metabolism 2008; 9: 581-591.
11. Dai J and Mumper R, "*Plant phenolics: extraction, analysis and their antioxidant and anticancer properties*", Molecules, 2010; 15: 7313-7352.
12. Mukherjee PK and Wahile A, "*Integrated approaches towards drug development from*
13. *Ayurveda and other Indian system of medicines*", Journal of Ethno pharmacology 2006;103:25-35. Ivanova A, Ikhova B, Batasalova T et al. "*New furostanol saponins from Smilax aspera L. and their in vitro cytotoxicity*", Fitoterapia 2011; 82:282-287.
14. Lalitharani S, Kalpana Devi V , Tresina Soris P et al. "*Ethno medicinal plants used by Kanikkars of Agasthiarmalai Biosphere Reserve, Western Ghats*", Journal of Ecobiotechnology, 2011;3: 16-25.

15. Tan RX, Zheng WR and Tang HQ. *"Biologically active substances from the genus Artemisia"*, Planta Medica 1998; 64 (4): 295-302.

16. Ferreira JF, Luthria DL , Sasaki T and Heyerick A. *"Flavonoids from* Artemisia annua *L.as Antioxidants and Their Potential Synergism with Artemisinin against Malaria and Cancer"*, Molecules 2010; 15: 3135-3170.

17. Wang J, Lijun H , Yuanyuan , Lei L et al. *"Hispidulin, a small flavonoid molecule, suppresses the angiogenesis and growth of human pancreatic cancer by targeting vascular endothelial growth factor receptor 2-mediated PI3K/Akt/mTOR signalling pathway"*, Cancer Science 2011; 102 : 219–225.