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# CYTOTOXICITY ACTIVITY OF AQUEOUS EXTRACT OF SOLANUM VIRGINIANUM ON HUMAN BREAST CANCER CELL LINE


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**ABSTRACT:**

**AIM:** The objective of the study was to examine the cytotoxic activity of aqueous extract of the *Solanumvirginianum* on MCF-7 human breast cancer cell line.

**METHODS:**

150g of *S.virginianum* was soaked in double distilled water and kept at 37°C for 3 days. The solution prepared was filtered. Fine filtrate was subjected to rota evaporate and 3g of plant extracted sample was obtained. The cytotoxic potency of *S. virginianum* was carried out by MTT assay against the above mentioned cell line.Furthermore, the morphological changes were analysed using phase contrast microscopy

**RESULTS:**

The aqueous extract of s.virginianum showed the cytotoxic potency against the MCF-7 cell line which confirmed with greater morphological changes upon 24 hrs treatment. The MTT assay clearly showed that the s.virginianum treatment has significantly reduced the cell viability when the concentration was increased for 24hrs.

**CONCLUSION:** The present study shows 50% cytotoxic effect at 30 µg/mL of aqueous extract of solanumvirginianum against MCF-7 human breast cancer cell line.


**Keywords:** Cytotoxicity, MCF-7, *Solanumvirginianum*, MTT assay.

## INTRODUCTION:

Cancer is a significant public health concern and is officially listed as the third contributing to death from infectious and cardiovascular diseases. Breast cancer is the second most continuous cancer in the world and the first most prevalent cancer in women (Parkin, Maxwell Parkin and Fernandez, 2006). According to the World Health Organization, there were 18.1 million new cases of cancer and 9.6 million deaths in 2018 and expected it reaches upto 29.5 million in 2040. Environmental and lifestyle modifications that are responsible for cancer includes ionizing radiation, hormonal therapy, reproductive behaviours of women, alcohol consumption, other dietary factors, obesity, lack of physical activity (Ferrini*et al.*, 2015). More than 2/3 rds of breast cancer cases are diagnosed in women whose ages range more than 50 years, and the majority of cases are from developed countries. Various genetic and environmental factors, those co existing, increase the risk of morbidity and reactivation of mammary gland cancer. Other commonly recognised and documented risk factors include age and cancer burden in family. Inflammatory Breast Carcinoma (IBC) is a rare and aggressive disorder. Specific entity of locally advanced inoperable breast cancer (LABC) is mainly due to the occurrence of erythema and dermal oedema over a substantial breast area (Castelló*et al.*, 2015). Histopathology has recognised breast cancer which is a heterogeneous disease that has both inter and intra tumour variability (Podkowa*et al.*, 2014). Advances in molecular research and in genetic sequencing are further recorded with next-generation DNA sequencing. The "inflammatory LABC breast carcinoma of the skin" is known as "secondary inflammatory breast carcinoma"(Orecchioni*et al.*, 2015). Patients with LABC must be treated with combination therapy using systematic and loco-regional methods and involve a well-coordinated clinical program and tight collaboration between medical, surgical and radiation oncologists(Thompson *et al.*, 2015).

Primary protection is to eliminate the conditions that contribute to the outbreak of the disease and to increase or enhance the immune response of the population. Secondary protection aims at terminating the process of disease development and may prevent the development of malign tumour. Mammography is used in breast cancer and

colonoscopy is used in colon cancer (Weigel*et al.*, 2016). Several treatments have recently been suggested for cancer therapy, all of which use plant-derived materials. Medicines have often played an important part in public wellbeing. Health medicinal plants offering a different field in drug research. Solanumvirginianum is also called as a Indian night shade or yellow berried night shade plant. It belongs to the family solanaceae and kingdom plantae. Medicinal properties of S. virginianum are used to treat whitlow, cough, asthma and chest pain. About 8000 metric tons of roots are used annually in ayurvedic industry in Maharashtra (Neef*et al.*, 2012). In an appropriate manner, the plant can be used as an effective agent against microbial pathogens and oxidative damage and to control insect vectors that propagate several diseases. The literature survey revealed that, no much work has been done to find out the antioxidant and anticancer activities of S. virginianum. The plant shows more importance due to the presence of several classes of medicinally important alkaloids along with potential antioxidant compounds (Mahato*et al.*, 2015).

Cancer, as one of the most lethal illnesses, calls for a systematic approach to prevention and recovery. Secondary metabolites from plants and microbes may be effective cancer drugs. Many plant chemical substances are toxic to cancer cells.which gave impacts on living systems as well as on other animals, and are therefore capable of becoming medicines. Our team has extensive knowledge and research experience that has translate into high quality publications (Rajeshkumar*et al.*, 2018; Nandhini, Rajeshkumar and Mythili, 2019; M. Gomathi*et al.*, 2020; Rajasekaran*et al.*, 2020; Vairavel, Devaraj and Shanmugam, 2020),(Santhoshkumar*et al.*, 2019),(Raj R, D and S, 2020),(Rajeshkumar*et al.*, 2018),(Saravanan*et al.*, 2018),(Gheena and Ezhilarasan, 2019),(Ezhilarasan, Sokal and Najimi, 2018),(Ezhilarasan, 2018)(Dua*et al.*, 2019; A. C. Gomathi*et al.*, 2020; Vairavel, Devaraj and Shanmugam, 2020),(Ramesh *et al.*, 2018; Duraisamy*et al.*, 2019; Ezhilarasan, Apoorva and Ashok, 2019; Arumugam, George and Jayaseelan, 2021; Joseph and Prasanth, 2021). So the aim of the research is to investigate the cytotoxic effect of aqueous extract of solanumvirginianum against human breast cancer cell line.

## MATERIALS AND METHODS:

### PREPARATION OF SAMPLE:

*Solanumvirginianum* powder was commercially obtained from IMPCOPS(Chennai, India). About 150g of sample was soaked in 500 ml of double distilled water and kept at 37°C for 3 days. The solution prepared was filtered with filter paper followed by whatmann paper. Fine filtered samples were concentrated by a rotary vacuum evaporator and the left-over solvent was evaporated to dryness using a hot air oven. 3g of material was obtained and immediately sorted at 4°C.

### CHEMICALS:

DMEM (Dulbecco's modified Eagle's medium), 0.25% Trypsin-EDTA solution, sodium bicarbonate solution, bovine serum albumin (BSA), MTT from Sigma Chemicals Co., St. Louis, USA. fetal bovine serum (FBS) and antibiotic/antimycotic solution, DMSO were purchased from Himedia, Sodium phosphate monobasic and dibasic, sodium chloride, sodium hydroxide, sodium carbonate, hydrochloric acid and methanol were purchased from Sisco Research Laboratories (SRL) India. The breast cancer cell line was procured from the National Centre for Cell Science (NCCS, Pune), India. The cells were grown in T25 culture flasks containing DMEM medium supplemented with 10% FBS.

### PREPARATION OF EXTRACT:

The required quantity of the extra was correctly weighed and dissolved in DMSO with concentration of 1mg/ml as a stock solution. This solution was subsequently diluted to a series of concentrations ranging from 20 to 300 µg/ ml.

### MTT ASSAY:

The cytotoxic effect of *S.virginianum* on MCF-7, were carried out MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay described by Koka(Koka*et al.*, 2018). Further, the viability of MCF-7 cells upon drug treatment was assessed by trypan blue exclusion test. Cells were seeded in 96-well plates at the density of $5X10^3$/100µl, after 24hrs cells were treated with different concentrations (0, 20, 40, 80, 100, 200 and 300 µg) of *S.virginianum.* After incubation, 20 µl of 5 mg/ml MTT stock solution was added to each well and incubated for 4 h at 37 °C. The obtained formazan crystals were solubilized with DMSO and the absorbance was measured at 570 nm using a microplate reader (SpectraMax M5, Molecular Devices, USA). Cell viability (%) has been shown as a ratio of absorbance (A570) in treated cells to absorbance in control cells (0.1 % DMSO) (A570). The IC50 was calculated as the concentration of sample needed to reduce 50 % of the absorbance in comparison to the DMSO-treated control. Percent cell viability was calculated following the equation:

$$\% \text{ of cell viability} = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

OD @ 570nm

**MORPHOLOGICAL STUDY:**
Based on MTT assay we selected the $IC_{50}$ value of Solanumvirginianum for further studies. The characterisation of morphological changes in breast cancer cells before and after treatment with Solanumvirginianum were observed under phase contrast microscope.

**STATISTICAL ANALYSIS:**
All data obtained were analyzed and computed statistically (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) using one-way ANOVA. Post-hoc testing was performed for inter comparisons using the LSD. In all tests, the level of statistical significance was set at $p<0.05$.

**RESULTS:**

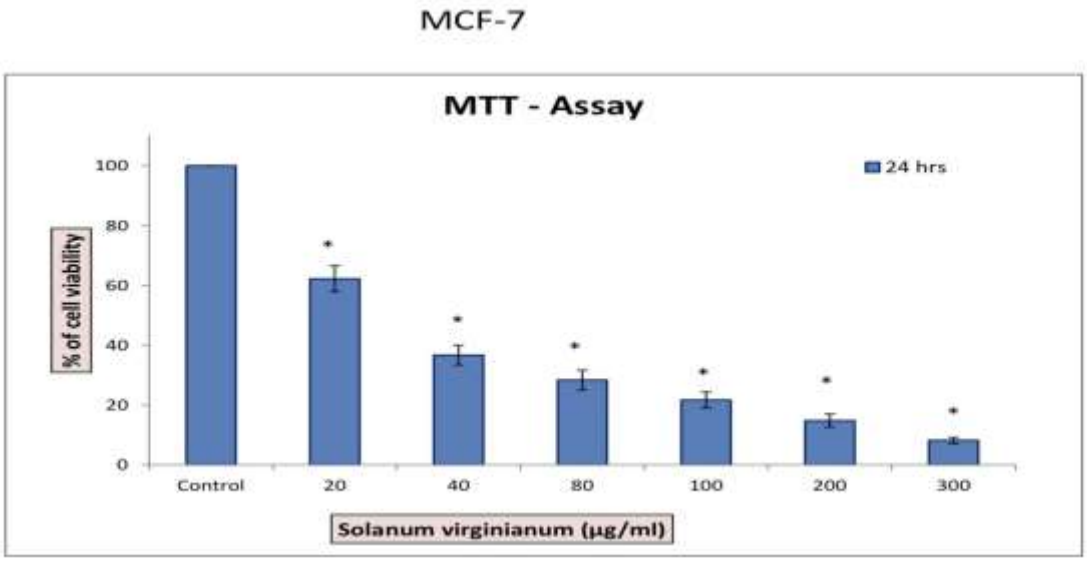


Figure 1: The cytotoxic effects of S.virginianum on MCF-7 cells was determined by MTT assay. The Cells were treated with different concentrations (0, 20, 40, 80, 100, 200 and 300 µg) for 24hrs. The 50% of inhibition observed in concentration of 30 µg/ml, which has been taken as $IC_{50}$ value and fixed for further experiments.
Data are shown as means ± SD (n = 3). * compared with the control-blank group, $p < 0.001$.

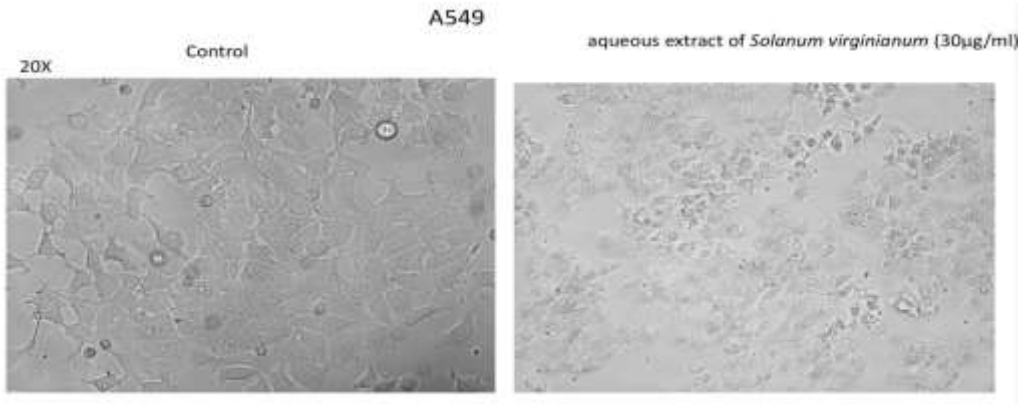


**Figure 2:** Represents the morphological changes in breast cancer cell line upon without and with treatment of *S.virginianum* at 30 µg/mL for 24hrs by phase contrast microscope at 20x magnification.

**DISCUSSION:**
Medicinal plants are contested as an alternative drug for cancer prevention and/curation in many nations around the world. A greater therapeutic effect of the above plant derived active compounds will be found to be an effective anti cancerdrug againstcancer cells.. Plants also have the opportunity to supply newer therapies, making them a potentially rich source of novel anticancer substances. Recently, numerous treatments have been proposed for cancer, which use plant-derived materials. Any herbs and plants include chemicals that influence human physiology, and these bioactive compounds include tannins, sugars, hormones, and flavonoids(Rajeshkumar*et al.*, 2019)(Rajeshkumar, Lakshmi, *et al.*, 2020)(Rajeshkumar, Tharani, *et al.*, 2020b)(Lakshmi *et al.*, 2017)(Lakshmi *et al.*, 2017; 'Phyto-assisted synthesis of zinc

oxide nanoparticles using Cassia alata and its antibacterial activity against Escherichia coli', 2019)(Rajeshkumar, Sivaperumal, *et al.*, 2020)(Rajeshkumar, Tharani, *et al.*, 2020a)(R. Jagadheeswari*et al.*, 2020)(*Molecular docking analysis of compounds from Lycopersiconesculentum with the insulin receptor to combat type 2 diabetes*, no date)(*Anticancer effects and lysosomal acidification in A549 cells by Astaxanthin from Haematococcuslacustris*, no date)(Akshayaa*et al.*, 2020)(Rajeshkumar, Agarwal, *et al.*, 2020)(Thangavelu*et al.*, 2020)(*Cytotoxic potentials of silibinin assisted silver nanoparticles on human colorectal HT-29 cancer cells*, no date)(Shaker Ardakani*et al.*, 2021)(Hashim*et al.*, 2021)(Krishnan and Lakshmi, 2013).

The compounds that inhibit cancer initiation are traditionally termed as blocking agents, this bioactive component present in plants can prevent carcinogenesis by blocking metabolic activation, increasing detoxification, or providing alternative targets for electrophonic metabolites (Anandakumar*et al.*, 2012). They may act by preventing the interaction between chemical carcinogens or endogenous free radicals and DNA, thereby reducing the level of damage and resulting mutations which contribute not only to cancer initiation but also progressive genomic instability and overall neoplastic transformation (Raghunandhakumar*et al.*, 2013). Bioactivity-guided fractionation of a 90 % methanolic extract of Solanumelaeagnifolium compound displayed cytotoxic activity against breast cancer cell lines (MCF7) with IC50 values of 5.2 µM, respectively (Radwan*et al.*, 2015). Whereas, the IC50 value of the present study varies from previous literature. The quantitative contents of compounds showed different concentrations among three plants, the highest concentration of alkaloids was P. sabiniana leaves, and the less concentration was Ferocactus sp. leaves.,(Paramasivam*et al.*, 2015). In-vitro short term cytotoxicity assays by DAL cells against seed extract of SolanumVirginianum was plotted by dose - response curve and showed more cytotoxic and anticancer activity (Tr*et al.*, 2017). Petroleum ether and chloroform extracts of Pinus and E. camaldulensis showed a promising anticancer activity (Abdulhamid*et al.*, 2013). Similar studies have found that the methanolic extract of S. virginianum had cytotoxicity and antitumor activity against Ehrlich's ascites carcinoma (EAC) in Swiss albino mice (Al-Bogami, Saleh and Moussa, 2018). Some compounds, on the other hand, had toxic activity to increase proliferation cancer cells at low concentration of an aqueous extract This result was in agreement with the previous studies which revealed the aqueous extract of natural herbs that had a side effect lead to the toxicity (Lu *et al.*, 2012). Such as piperine lead to neurotoxicity, immunotoxicity, and reproductive toxicity have been reported (Dogra, Khanna and Shanker, 2004), and hepatotoxicity and embryonic toxicity can also be induced by sanguinarine(Chan, 2011).

This activity of inhibition may be due to the nature of the compounds found in each crude extract and their interaction with metabolic nature of each type of cancer cells or may be due to the effectiveness of some enzymes that act as antioxidants especially in cancer cells (Asokkumar*et al.*, 2012). The limitation is that the study does not involve any in vivo study(Ezhilarasan, Apoorva and Ashok, 2019)(Danda, Krishna, *et al.*, 2010)(Ramaduraii*et al.*, 2019)(Sathivel*et al.*, 2008)(Panda *et al.*, 2016)(Neelakantan*et al.*, 2012)(Govindaraju, Neelakantan and Gutmann, 2017)(Sekhar, Narayanan and Baig, 2001)(DeSouza*et al.*, 2014)(Nasim*et al.*, 2010)(Danda, Muthusekhar, *et al.*, 2010)('Molecular structure and vibrational spectra of 2,6-bis(benzylidene)cyclohexanone: A density functional theoretical study', 2011)(Putchala*et al.*, 2013)(Neelakantan, Grotra and Sharma, 2013)(Suresh *et al.*, 2014)(DeSouza*et al.*, 2014), So its effect is not assessed. This paves way for various future studies such as to view the drug action in in vivo studies and also to know about the side effects of the extract.

## CONCLUSION:

The present study shows the inhibitory concentration percent as 30 µg/mL, 50% of the cells and it is concluded that the aqueous extract of Solanumvirginianum had a slight effect on the cell viability of breast cancer cell lines. In other words, it has cytotoxic activity against MCF-7.

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## CONFLICT OF INTEREST:

Nil

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