

IN VITRO EVALUATION OF METHANOLIC EXTRACT OF SCHEFFLERA VENULOSA FOR CYTO- COMPATIBILITY.

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ABSTRACT

The present work gives an insight of cytotoxic activity of the plant extract. *Schefflera venulosa*, which has been for its extensive use in the folk medicine. It is loaded with phyto-chemicals such as saponins, tannins, flavonoids, alkaloids, cardiac glycosides and reducing sugars. They bear the major activities like anti-inflammatory, anti-oxidant, Hepatoprotective. This present research work highlights the cyto-compatible nature of *S.venulosa* methanolic extract on human cells.

Objective:- To study the cyto-toxic effects of the methanolic leaf extract of *S. venulosa*. **Methods:-** The methanolic extraction of the plant leaf was obtained by standard methods. The Cytotoxicity of the

plant extract was evaluated by performing MTT assay over MCF-7 (Epithelial Breast Cancer cell line) cell line. **Result:-** The methanolic extract showed cytotoxicity effect of *S.venulosa* on MCF-7 cell line by inhibiting MCF-7 growth/proliferation. The estimated methanolic extract showed moderate growth inhibition of MCF-7 cells at 500µg/ml. **Conclusion:-** The study concludes, the leaf sample contains active compounds which are moderately cyto-toxic on the cancer cells, which was confirmed by the significant inhibition of cancer cell's growth and killed them to certain extent in higher concentrations.

KEYWORDS: Cytotoxicity, MCF-7, MTT assay, *Schefflera venulosa*, Methanolic Extract.

INTRODUCTION

S.venulosa is an evergreen shrub or a small tree found in the tropical evergreen forest. It was named to honour the scientist Johann Peter Ernst Schefflera, a botanist from Warsaw. Genus was named as *Schefflera* and species *venulosa* means veins in Latin. This plant has Henna

like aroma and has been known to have 600 species diversified around specific parts of world.^[1,2] Majorly found in the Western Ghats of India and in some parts of Taiwan, Cambodia, Andaman & Nicobar. It is often found along the river banks and in the coastal area of mangrove vegetation.^[3] This plant can grow up to 10 meters tall, sometimes it is epiphytic and sometimes it adopts for climbing habitat. Inflorescences bearing umbellules, heads, racemules or spicules and more or less fleshy fruits. Along with the ornamental uses of *S.venulosa* and it was proved to have various medicinal properties, traditionally known to have wound healing property as stated by the local people.^[4] For the betterment of Human life and to increase life span, attempts have been made to explore the nature. Studies such as Ayurveda, Unani, Sidha, Amchi have also been exploring the important medicinal values of the plant and converting them into the medicinal products. Majority of the work has been reported on leaf extracts for their medicinal properties.^[5] The chemical examination of the *S.venulosa* plant extract revealed anti-bacterial, anti-oxidant, anti-inflammatory activity.^[6] Since it has been found to be having many medical application as said above further research may lead to the production of noble medicinal compounds. It is used in promoting blood circulation to disperse blood clots, improving microcirculation and treating acute myocardial ischemia, coronary heart diseases. The Bark of the plant is used to cure the cough. The decoction made by leaves is used in aromatic baths or Herbal baths. The roots are mixed with rice to cure DROOPSY. Wood of the plant is chewed to cure tooth ache. The lecithin extract from the plant has a good wound healing property.^[7,8]

Cancer is a condition which is characterized by the presence of mass of uncontrollable cells which are malignant in nature. Breast cancer is a second leading type of cancer in woman, causing severe morbidity in the world.^[9] Presently there are different treatment methods are being employed such as radiotherapy, chemotherapy and operative procedures and combined therapies.^[10] There are different types of breast cancers identified such as adenocarcinoma which originate in lobules and ducts and sarcoma of the muscle cells, fat cells.^[11] Different stages of breast cancer have been characterized based on strategies such as size of the tumor, invasiveness, presence of tumor cells in lymph nodes and metastasis.^[12,13]

Hereby, we proposed to study the cyto-compatible nature of *S.venulosa* extract (methanolic) on breast cancer cell line (MCF-7).

MATERIALS AND METHODS

Plant Collection

The leaf material of *S. venulosa* was collected from the natural habitat from Western Ghats in the month of May 2017. Leaves were washed under running water and shade dried for about 5-6 days to remove the moisture content. Dried leaves were grinded into coarse powder and packed into a sterile sealed container for the further use.

Extraction

Powdered leaves were used to obtain methanolic extract by warm extraction. Briefly, dried leaf powder was soaked in methanol and kept for stirring for 48-72 hours. Later methanolic mixture was filtered using filter paper and collected into a sterile glass beaker. Filtered mixture was kept for drying, during which it was loosely covered with aluminium foil. After the methanolic remnants are evaporated, the extract was used for experiments.

Cell Culture

The cells of the MCF-7 (Epithelial cells of breast cancer) were purchased from NCCS (National Center For Cell Sciences) Pune. Cells were nourished with DMEM-HG media and kept for incubation at 37°C, 5% CO₂ until they reach confluence. Upon the cells reaching 80-90% confluency, cells were passaged. After passaging cells were used for experiment. MCF-7 cells were seeded in 96 well plate at 2.2x10⁴ cells/well. Cells were allowed to attach and grow for 24 hours.

Number of wells in 96 well plate was considered depending on experimental setup and concentrations of plant extract considered. Five different concentrations of plant extracts were considered to test the cyto-compatibility. Standard drug (Camptothecin) and untreated cultures were considered as control groups. Experiments were performed in duplicates.

The seeded cells observed under microscope and confirmed the attachment of cells and formed a monolayer.

MTT ASSAY

After 24 hours of drug treatment cells were taken for performing MMT assay and cytotoxic effects of drug on MCF-7 cells was done. Briefly, cells were treated with pre-defined concentration of plant extract and controls for 24 hours. Later, 0.5mg /ml MTT was added to each well and incubated for 3 hours. Post MTT incubation, culture plate was observed under

microscope to check the needle like formazon crystals formation. Formazon crystals were dissolved by adding DMSO to cells and supernatant media was read for optical density at 625nm using spectrophotometer. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC₅₀) values is generated from the dose-response curves for each cell line.^[14]

$$\% \text{ Growth Inhibition} = 100 - \left\{ \frac{100 \text{ Mean OD of individual test group}}{\text{Mean OD of control group}} \right\} \times 100$$

RESULTS

Cytotoxic activity was performed on the MCF-7 cell line, the results were obtained from the Spectrophotometer analysis at 625 nm and are as above. The standard drug (Camptothecin) shows the cell viability count of 36.879% where almost of the cancer cells are dead and the remaining were in the stage of apoptosis. The plant drug which was treated on MCF-7 cell line also showed some of cytotoxic activity. The cytotoxic activity was very negligible in the first 3 (100,200,300 µg/ml) concentration and the major activity was observed in the 4th & 5th (400,500 µg/ml) concentration. The test drug showed the cytotoxic effect and the cell viability was 87.517%, 78.581%. Thus, some compounds of the plant extract to be cytotoxic effect on cancer cells which could be analysed further by the sequential extraction of the plant leaf. (Figure 1).

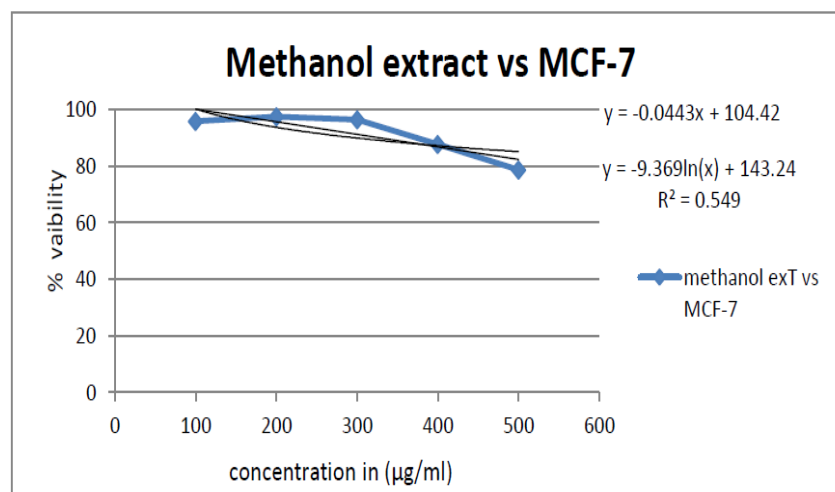


Figure 1: Cytotoxic activity of *S.venulosa* on MCF-7.

DISCUSSION

The leaves of *S. venulosa* are used for various medicinal purposes recent studies have revealed that the plant has anti-oxidant and cardio-protective activity.^[14,15,16] Therefore the aim of the

present work is to evaluate the cytotoxicity of the methanolic extract of *S.venulosa*. Traditional medicines are effective against many diseases and are based on their use in folklore medicine system. Natural medicine contains highly potent bioactive compounds and therapeutic properties, so they play an important role in the treatment of various human diseases.

The present study revealed that the methanolic extract of the plant *S.venulosa* showed cytotoxic activity on the cancer cell line MCF-7. The activity is due to the presence of various phytochemicals in the methanolic extract. These phytochemicals such as Flavonoids and Polyphenols have cytotoxic potential. The cytotoxic effect shown by *S.venulosa* can be used in production of noble compound by pharmaceutical industry to harness its useful biological activity and produce many drugs which will be helpful for human race. A further challenging aspect is related to the development of efficient Pharmaceutical compound. Further studies are necessary to isolate the constituent responsible for the anti-cancer activity.

CONCLUSION

The anti-cancer study of the methanolic leaf extract *S.venulosa* which has been tested on MCF-7 cell line by MTT Assay cell growth inhibition. The maximum cell were inhibited or killed at the 500µg/ml concentration. By which the study reveals that the leaf sample contains active compounds which are moderately cyto-toxic on the cancer cells. The study showed that the methanolic leaf extract has significantly inhibited the growth of the cancer cell and killed them to certain extent. Thus methanolic extract of the *S.venulosa* could be used as potent anti-cancer agent for the cure of Breast Cancer.

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CONFLICT OF INTEREST

We declare no conflict of interest in publishing this data.

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