Evaluation of Anti – cancer Activity of Ethanolic Extract of *Spinacia oleracea* by High Throughput Screening

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**ABSTRACT**

Environment became polluted, due to that human beings are getting so many diseases but nature has provided as a good sources of medicine as a remedy. In that course *Spinacia oleracea* is a leafy vegetable. It has so many pharmacological effects like Anti-oxidant, Anti-proliferative, Anti-inflammatory, Anthistaminic, CNS depressant, Protection against gamma radiation. In this study the anti-cancer activity of this plant was evaluated by High Throughput Screening. The Sulfoquinovosyl Diacylglycerol is responsible for anti-cancer activity. The ethanolic extract of *Spinacia oleracea* shows cell lysis in cancer cell lines. Thus it acts as a good source of anti-cancer activity.

**Keywords:** Anti cancer activity, *Spinacia oleracea*, High Throughput screening, MTT assay, cell lysis etc.

**INTRODUCTION**

In Present scenario, Cancer is one of the greatest killers worldwide and is spreading promptly. Cancer is the third leading cause of death worldwide, preceded by cardiovascular and infectious diseases. It is a generic term for a group of more than 100 diseases that can affect any part of the body1. In spite of the many advances in cancer treatment, chemotherapy for solid tumours is still greatly limited by a lack of selective anti-cancer drugs and by the recurrence of drug-resistant tumours; finding a source of novel chemo therapeutics continues to be a focus of effort2. Various plant parts are extracted for the treatment of cancers. Diets rich in vegetables are known to reduce cancer risk, implicating edible plants as potential sources of anti-cancer agents3.

**MATERIALS AND METHODS**

**Plant Material**

The *Spinacia oleracea* was collected from Malviya Nagar, Jaipur. The botanical identification was carried out by local florists and finally confirmed with comparing the authentic specimens in Botanical herbarium, Rajasthan University, Jaipur, India. The plant material was shade dried and powder was used for extraction.

**Extraction**

100gms of the dried powder was weighed and extracted with 200ml of alcohol in Soxhlet Apparatus. The process was continued for 1 week. The extract was concentrated by evaporating the alcohol. The deep green colour extract was obtained4.

**Cell lines**

The cell lines were obtained from NCCS Pune. The cell lines used for the study were A 549, Hela, K 562, MDA MB, Hep G2, which represents lung, cervix, bone, breast and liver Cancers respectively.

**Procedure**

**MTT Assay**

MTT(3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) stock solution was prepared by using 5 mg of MTT medium was dissolved in 1 ml of Phosphate buffer solution5.

**Cell culture**

The cell lines were maintained in 96 wells micro titer plate containing MEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamicin (10µg), Penicillin (100 Units/ ml) and Streptomycin (100µg/ml) in presence of 5% CO2 at 37°C for 48-72 hours6.

**Cytotoxic Assay**

In vitro growth inhibition effect of alcoholic extract of *Spinacia oleracea* was assessed by calorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells.

The supernatant was removed from the plates and fresh MEM solution was added and treated with different concentrations of extract diluted with DMSO. Control group contains only DMSO. The serial dilutions were prepared by starting concentration of 300 µl of pure extract. The next dilutions were made with DMSO.

The final concentrations were 300, 150, 75, 37.5, 18.75, 9.37, 4.68, 2.34, 1.17, 0.58µg/ml. After 48hrs incubation at 37°C in a humidified atmosphere of 5% CO2, stock solution of MTT was added to each well (20µl, 5mg per ml in sterile PBS) for further 4 hr incubation. The supernatant carefully aspirated, the precipitated crystals of “Formazan blue” were solubilised by adding DMSO (100µl) and optical density was measured at wavelength of 570nm by using LISA plus7.
RESULTS AND DISCUSSION

The anti-cancer activity of Spinacia oleracea was performed by the MTT Assay. The results obtained were as follows.

The ethanolic extract of S. oleracea was subjected to test different cell lines to determine IC50.

The IC50 values reveal that the ethanolic extract of S. oleracea produced the anticancer activity against lung and bone cancer (A549 cell lines 236 µg and K562 cell lines 226 µg) among all the cell lines.

CONCLUSION

The studies conclude that S. oleracea is having anti-proliferative activity and our study has revealed that the ethanolic extract of S. oleracea shows the anti-cancer activity against lung and bone cancer when tested on different cell lines.

Reasons for the difference in the study results are primarily the plant S. oleracea, which was used in our study was naturally grown product of medicinal gardens and in other reviewed studies the plant was procured commercially from the market.

Secondarily the research work was carried out at geographically different regions so there might be a possible chance of developmental difference expressed in terms of chemical constituents, thirdly different cell lines were selected in the various reviewed studies which is also may be another reason for different in the outcome of the studies.

REFERENCES


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