Research Article

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

Rajesh Asija, Radheyshyam Kumawat, Deepak Sharma, Preethi Sagar*
Maharishi Arvind Institute of Pharmacy, Jaipur, India.
***Corresponding author's E-mail:** preethirose001@gmail.com

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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-infammatory, Antihistaminic, 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 body[1]. 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 effort[2]. 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 agents[3].

MATERIALS AND METHODS

Plant Material

The *Spinacia oleracea* was collected from Malviya Nagar, Jaipur. The botanical identification was carried out by local floras 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 obtained[4].

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 G_2 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 solution[5].

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 (10ug), Penicillin (100 Units/ ml) and Streptomycin (100µg/ml) in presence of 5% CO_2 at 37°C for 48-72 hours[6].

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 plus[7].

Graphs

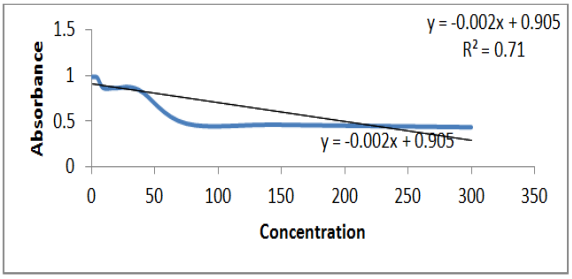

Figure 1: DRC of A549

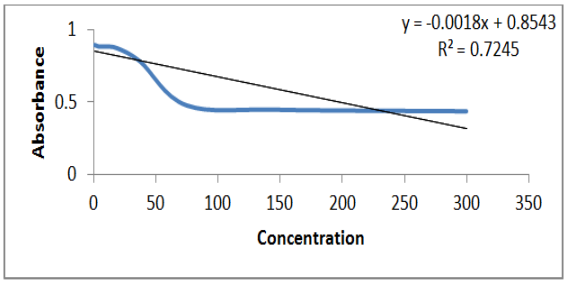

Figure 2: DRC of Hela

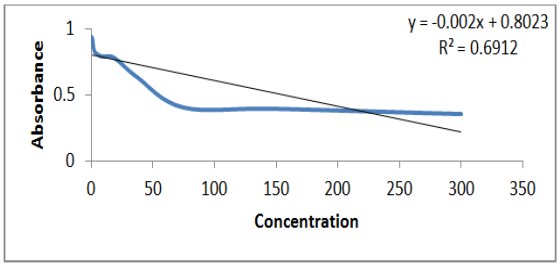

Figure 3: DRC of K 562

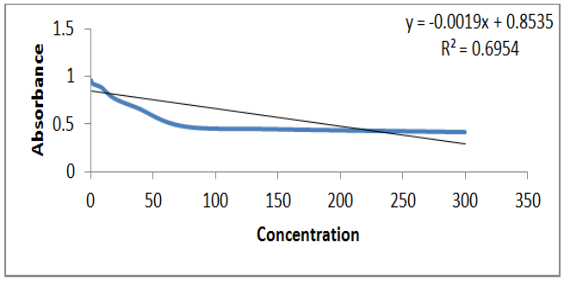

Figure 4: DRC of MDA MB

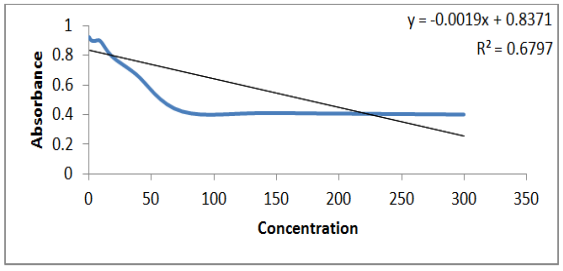

Figure 5: DRC of Hep G_2

RESULTS AND DISCUSSION

The anti - cancer activity of *Spinacia oleracea* was performed by the MTT Assay[8]. The results obtained were as follows.

Table 1

Cell lines	IC_{50} values	Concentration tested (µg/ml)
A549	236 µg	300, 150, 75, 37.5, 18. 75, 9.37, 4.68, 2. 34, 1. 17, 0. 58.
Hela	414 µg	300, 150, 75, 37.5, 18. 75, 9.37, 4.68, 2. 34, 1. 17, 0. 58.
K562	226 µg	300, 150, 75, 37.5, 18. 75, 9.37, 4.68, 2. 34, 1. 17, 0. 58.
MDA-MB	433 µg	300, 150, 75, 37.5, 18. 75, 9.37, 4.68, 2. 34, 1. 17, 0. 58.
HepG2	437 µg	300, 150, 75, 37.5, 18. 75, 9.37, 4.68, 2. 34, 1. 17, 0. 58.

The ethanolic extract of *S. oleracea* was subjected to test different cell lines to determine IC_{50}.

The IC_{50} values reveal that the ethanolic extract of *S. oleracea* produced the anticancer activity against lung and bone cancer (A 549 cell lines 236 µg and K 562 cell lines 226 µg) among all the cell lines[9].

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[10].

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[11].

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Corresponding Author's Biography : K. Preethi Sagar

K. Preethi Sagar, pursuing M. Pharmacy specialization in Pharmacology in Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan. I wrote research article by the title "Evaluation of anti cancer activity of ethanolic extract of *Spinacia oleracea* by High Throughput Screening".