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INVITRO ANTICANCER ACTIVITY OF *SPINACIA OLERACEA* AGAINST VARIOUS MAMMALIAN CELL LINES

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ABSTRACT

Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression. In this study the anticancer potentials of plants was investigated against MCF-7 and HeLa cells *in vitro*. Cytotoxicity of various plant extracts was determined by MTT assay. The results showed that the methanolic extract of Indian spinaches *Spinacia oleracea* possessed a moderate amount of anticancer activity and the IC_{50} value was recorded. The most potent anticancer activity was observed with the methanolic bulb extract of *Spinacia oleracea* with IC_{50} values of 45.9µg/ml and 47.9µg/ml on MCF-7 and HeLa cells respectively. Phytochemical analyses revealed the presence of large amount of alkaloids and flavonoids in the potent plant extracts which may be suggested to play an important role in their anticancer activities.

KEYWORDS: Anticancer, cytotoxicity, MCF-7, HeLa cells, MTT assay.

INTRODUCTION

Cancer is one of the major human diseases and causes large suffering and economic loss world-wide. Chemotherapy is one of the methods of treating cancer. However the chemotherapeutic drugs are highly toxic and have devastating side effects. Various new strategies are being developed to control and treat several human cancers (Modha and Modha, 2007). Over 60% of anticancer drugs available in the market are of natural origin. Natural products are also the lead molecules for many of the drugs that are in use (Cragg et al., 1997). Therefore, the phytochemicals present in several herbal products and plants may

have the potential to act as preventive or therapeutic agents against various human cancers (Modha and Modha, 2007). The increased popularity of herbal remedies for cancer therapy perhaps can be attributed to the belief that herbal drugs provide benefit over that of allopathy medicines while being less toxic (Gupta et al., 2004). Since the conventional therapies have devastating side effects, there is a continuous need for search of new herbal cures of cancer (Aquil et al., 2006).

Apoptosis, or programmed cell death, is one of the most finely coordinated regulatory functions for maintenance of the homeostasis in the living organism. It involves the continuous checking of the cellular integrity and cascade-like events of self destruction when the integrity of the organism is endangered. Morphological hallmarks of apoptosis are nuclear condensation, cell shrinkage, membrane blebbing and the formation of apoptotic bodies. These changes are accompanied by biochemical features, including DNA fragmentation and the proteolytic cleavage of a variety of intracellular substrates.

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in “Rigveda”, which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. The medicinal plants are being used since the human civilization for their ailments to cure various diseases and symptoms (Yogisha and Raveesha, 2009). Medicinal plants are known to produce various secondary metabolites which employ to develop new eco-friendly bio-pesticides and other pharmaceutical and nutraceuticals relevance (Bobbarala et al., 2009). Traditional medication would be the first hand medical practices all around the world by the healers about 80% world’s population (WHO, 1993).

Spinacia oleracea possess various nutritional factors such as vitamins, minerals and other phytochemicals which is very essential for day to day life (Tongco et al., 2015). Common spinach, *Spinacea oleracea* is well known that has been used as leafy vegetables. Apart from the various nutritious benefits of the plant there are lot of pharmacological potential have been reported worldwide (Mane et al., 2015). The present investigation was taken up for evaluating the antiproliferative potential possessed by the methanol extract of leaves of *S. oleracea* against various human cancer cell lines.

MATERIALS AND METHODS

Collection of plant samples

The leaves of the medicinal plants were used for the present study, the plants of *Spinacia oleracea* L was obtained from Coimbatore localities, Tamil Nadu, India. The plant parts were identified taxonomically and authenticated according to various literatures, Flora of Madras Presidency and Wealth of India including other pertinent taxonomic literature.

Phytochemical Analysis

The collected leaf samples were washed thoroughly two times with running tap water and once with sterile water, air-dried, powdered using a pulverizer and used for extraction. About 50 grams of air-dried and coarsely powdered plant material was extracted successively with 250 ml of methanol using a Soxhlet extractor for at least 15 reflux. After complete extraction, the extract in the round bottom flask were removed and condensed using rotary evaporator. After solvent evaporation, extracts were weighed for the percentage yield calculation. The thick syrup plant extract were labeled and stored at 5°C in sterile screw-capped vials for further use.

Preliminary phytochemical screening of methanolic extract of *S. oleracea* leaf was carried out to detect the phyto-constituents using standard conventional protocols (Trease and Evans, 1989; Sofowara, 1993; Harbone, 1998). Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Tumour cell lines

Cell lines of different tissue origin such as MCF-7 (human breast tumor) and HeLa (human cervical cancer) were used. Cells were cultured in minimum essential medium (MEM) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin and 50 µg/ml streptomycin in a humidified atmosphere of 5% CO_2 in air at 37^0C in a CO_2 incubator.

MTT assay (Mossman et al., 1983)

Antiproliferative effects were measured in vitro by using MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assays. After treatment, the living cells were assayed by the addition of 20 µl of 5 mg/ml MTT solution. Finally, the reduced MTT was assayed at 545 nm wells with untreated cells were utilized as controls. Antiproliferative and cytotoxic effects were distinguished by cell number and the duration of treatment (72 h, 5000 cells/w, and 24 h, 25000 cells/w, respectively). Stock solutions of

the tested materials were prepared with dimethyl sulfoxide (DMSO). The highest DMSO concentration (0.3%) of the medium did not have any significant effect on the cell proliferation. Extracts which demonstrated potent activity (growth inhibition > 50%) were selected for further in vitro testing (dose-response curve and cytotoxicity). To study the interactions between acridones and doxorubicin, a checkerboard method was applied. A series of 2-fold dilutions of the acridones was tested in combination with 2-fold dilutions of doxorubicin. The cell growth rate was determined with MTT staining drug interactions were evaluated according to the following system (fractional inhibitory index = FIX).

FIX < 0.5	Synergism	1 < FIX < 2	Indifferent effect
FIX = 0.51-1	Additive effect	FIX > 2	Antagonism

RESULTS AND DISCUSSION

Qualitative phytochemical analyses for alkaloids, carbohydrates, tannins, phenols, gums and mucilage, fixed oils and fats, saponins, proteins, volatile oils, flavonoids and steroids were screened in methanolic extracts of the selected spinach *S. oleracea*. The screening of the extract indicated the presence of alkaloids, tannins and saponin in the methanolic extracts of leaves (Table 1). Thick syrup were obtained the spinach *S. oleracea* was yielded as 2.26 g (4.52%). The plant extract yield percentage on the usage of methanolic extract agreed with the earlier reported by Jamuna *et al*. (2014) obtained in *Hypochaeris radicata* L. The plant extract obtained using soxhlet is varied among the herbal plants to plant. In a plant, different parts having differently yielded (Krishna et al., 2013).

Total phenol content was estimated using folinphenol reagent under standard assay conditions. It was estimated that, *S. oleracea* methanolic extract possessed 684.5 mg GAE/g of dry extract (GAE: gallic acid equivalent). The *S. oleracea* methanolic extract possess alkaloids, flavonoids, terpenoids, phenolic compounds, saponins and cardiac glycosides in their phyto-constituents. Although, these secondary metabolites possess various bioactive potentials have been demonstrated in various plants (Mohamed Zaky Zayed and Benedict Samling, 2016).

S. oleracea possesses most of the valuable and bioactive secondary metabolites with their phytoconstituents**.**

Table 1: Phytochemical screening of spinach plant methanolic extract.

Phyto-constituents	*S. oleracea*
Alkaloids	+
Flavonoids	+
Terpenoids	+
Phenolic Compounds	+
Saponins	+
Tannins	+
Glycosides	+
Cardiac Glycosides	+
Coumarins	-

+: presence; –: absence

Anticancer activity of *S. oleracea* was studied in different mammalian cell line. Anticancer activity of methanolic extract of *S. oleracea* as well as standard was determined through MTT cytotoxicity assay. In the preliminary study, the metahanolic extract showed the good yielding capacity of phytocompounds activity. In this regards, the present investigation the methanolic extract of *S. oleracea* was studied in HeLa and MCF-7 cell lines and its result labeled in the table 2 and also made with standard drug 5-Fluorouracil.

The minimum cell viability (22.7%) and maximum cell inhibition (83.7%) were noted in 200 µg/ml concentration of *S. oleracea.* The IC_{50} value (45.9µg/ml and 47.9µg/ml) was calculated for anticancer activity of methanolic extract of *S. oleracea* against HeLa and MCF-7 cell lines. The 5-Fluorouracil used as a standard for this study. In the standard, the minimum cell viability (18.4%) and maximum cell inhibition (86.7%) were observed in higher concentration. The percentage of cell inhibition was noted in the different concentrations of methanolic extract of *S. oleracea* ranges from 20 to 200 µg/ml. The lowest cell inhibition (33.4%) was recorded in the lowest concentration and highest cell inhibition (83.7%) was noted in the higher concentration of methanolic extract of *S. oleracea.*

Anticancer properties of many natural compounds isolated from different Indian plant extracts have been reported. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Plant-derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties, which include cytotoxic and chemopreventive effects

(Abdullaev, 2001). They were the first agents to advance into clinical use for the treatment of cancer (Cragg and Newman, 2005).

Table 2: Survival analyses of cancer cells treated with extracts of *S. oleracea.*

Concentrations (µg ml-^{1})	HeLa		MCF7	
	Cell viability (%)	Cell inhibition (%)	Cell viability (%)	Cell inhibition (%)
20	62.4	33.4	68.7	35.3
40	53.2	42.7	62.3	48.6
80	45.4	51.2	54.4	56.3
120	35.7	62.4	42.3	69.7
160	29.4	76.5	37.1	78.1
200	23.5	81.8	22.7	83.7
Vehicle control (DMSO)	100	0	100	0

In vitro cytotoxicity against four human cancer cell lines was determined by Monks et al. (1991) using 96-well tissue culture plates. One hundred microlitres of cell suspension was added to each well of the 96-well tissue culture plate. The cells were allowed to grow in a carbon dioxide incubator (37°, 5% CO_2, 90% RH) for 24h. Test materials in complete growth medium (100 µl) were added after 24 h of incubation to the wells containing cell suspension. The plates were further incubated for 48 h. The cell growth was stopped by gently layering trichloroacetic acid (50%, 50 µl) on top of the medium in all the wells. The plates were incubated at 4^{o}C for one hour to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water to remove trichloroacetic acid, growth medium low molecular weight metabolites and serum proteins and then air-dried. The plates were stained with sulphorhodamine B dye (0.4 % in 1% acetic acid, 100 µl) for 30 min. The plates were washed five times with 1% acetic acid and then air-dried (Skehan et al., 1990).

Withania somnifera as a potential source of new molecules that can curtail cancer growth were studied by Dredge et al. (2003). *S. oleracea* have also been shown to inhibit the growth of human cancer cell lines comparable to that produced by 5-Fluorouracil. The bulb extract produced antiproliferative activity on MCF-7 (human breast tumor) and HeLa (human cervical cancer) tumor cell lines. The inhibitory concentrations obtained was 25.1±0.91 against colon cell line HCT-116 (Jayaprakasam et al., 2003), but in this study bulb extracts from different cancer cell treatments of *S. oleracea* cultivated in fly ash containing soil had

shown more than 82% inhibition against human cell lines. Furthermore this study has reported growth inhibitory importance in *S. oleracea* against various cancer cell lines i.e. MCF-7 and HeLa tumor cell lines. Hence, this study has revealed remarkable anticancer potential in the leaves of *S. oleracea*.

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