



Cytotoxic Activities of *Salvia Sahendica* Extract on HeLa Cells

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Abstract. The study was aimed to evaluation of the anticancer activity of the *Salvia sahendica* on the HeLacell line. The cytotoxicity of *S. sahendica* on HeLacell was evaluated by the MTT assay. Results showed that *S. sahendica* ethanolic extract has significant cytotoxic effect on HeLacell line in concentration range between 0.156 mg/ml to 1.25 mg/ml. Maximum cytotoxic effects were found in HeLa cells after incubation with the *S. sahendica* extract at 0.625 mg/ml (68.02%). Also results showed the concentrations producing 50% growth inhibition (IC₅₀) of the *S. sahendica* extracts was 3.45 mg/ml. The *S. sahendica* extract was found dose inhibits the proliferation of cancerous HeLa cells possibly causes through rich secondary methabolits. Based on the results of this experiment extract of *S. sahendica* has cytotoxic effect on HeLa cell line, and could be usefull in variety fields as the anticancer complex.

Keywords: *Salvia sahendica*, ethanolic extract, cytotoxic, MTT assay

1. INTRODUCTION

Cancer is major health problem in both developed and developing countries. Cancer after cardiovascular disease is the second leading cause of death. It is the abnormal growth of cells in our bodies and is a complex genetic disease that is caused primarily by environmental factors, that can lead to death. Because of high death rate associated with cancer and because of serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative complementary methods of treatment.

Plants have been used for treating diseases since time immemorial. Species of the genus *Salvia* (Lamiaceae) are well known throughout the world for their medicinal properties which have made it an attractive choice for many researchers. Although some *Salvia* species have been scientifically studied in many parts of the world and are reported to have various biological activities including antibacterial (Ulubelen *et al.*, 2001), anti-oxidant (Couladis *et al.*, 2003), anti-inflammatory (Perry *et al.*, 2003), anticancer (Liu *et al.*, 2000) and anticholinesterase (Perry *et al.*, 2003), but there is little scientific basis to validate the claims by traditional medicine practitioners about the effectiveness of local indigenous *Salvia* species. A literature search indicated that the genus *Salvia* has been a popular topic in phytochemical and ethnobotanical research. The solvent extracts, essential oils and compounds isolated from *Salvia* species revealed that they displayed a broad range of pharmacological properties, both *in vitro* and *in vivo*. *Salvia sahendica*, also known as Maryam Goli sahandy, is one of the native plants used to Persian medicinal herbs. From 900 *Salvia* species which are distributed in the world, about 17 species are endemic of Iran (Mozafarian, 1996). *S. sahendica* extract (endemic of Iran) is traditionally used for antibacterial, anti-fungi proposes and treatment of dyspepsia in many part of Iran (Lotfipour *et al.*, 2007). In addition, the essential oils and various extract of *S. sahendica* were found to have antioxidant potential (Salehi *et al.*, 2007).

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The objective of this study was to examine the in vitro cytotoxic activities of ethanol standardized extract, on HeLa cell lines, using a MTT cytotoxicity assay.

2. MATERIALS AND METHODS

2.1. Preparation of plant extract

Salvia sahendica plants were collected from the west part of Iran (tabriz) in April 2013. Mr. Eslami from the Department of Biology of Qaemshahr, Iran identified the plant. The whole parts of the plant were shade dried and grinded into powder. Extraction of ethanolic extract was carried out by macerating 40 g of powdered dry plant in 120 ml of 96% ethanol for 48 h at room temperature. Then, the macerated plant material was filtered through Whatman filter paper (NO.4) and dried under reduced pressure at 37 °C with rotator evaporator and then evaporated to dryness. Briefly, the concentrated plant extracts were dissolved in dimethyl sulphoxide (DMSO) (SIGMA, USA) to obtain appropriate solutions of the extracts. The sub-stock solutions of 0.156, 0.312, 0.625, 1.25, 2.5, 5, 7.5 and 10 mg/ml was prepared by diluting the stock solution into serum-free culture medium, RPMI 1640 (the percentage of DMSO in the experiment should not exceed 1). The stock and sub-stock solutions were both stored at 4 °C.

2.2. Cell cultures

Human cervical cancer cell line (HeLa) were purchased from Pasteur Institute of Iran (NCBI C115). The cells was grown and maintained in a humidified incubator at 37 °C and in 5% CO₂ atmosphere. RPMI-1640 medium (SIGMA) was supplemented with 0.01 mg/ml heat inactivated Fetal Calf Serum (FCS), 100 units/ml penicillin, and 100 µg/ml streptomycin (ALL FROM INVITROGEN GIBCO) was used for cell cultures. Upon reaching confluency, the cells were passaged. After being harvested from sterile T75 culture flasks, the cells were counted using a hemocytometer and cell viability was determined by trypan blue exclusion. Three thousand cells from log phase cultures were seeded in 100 µl of RPMI medium supplemented with 10% fetal bovine serum per well of 96-well flat-bottom culture plates (Phelan, 1998). Cells were incubated with the *S.sahendica* extract for a defined time (72 hours). Proliferative response and cell death of the *S.sahendica* extract-treated cells were determined using MTT assay and cell death ELISA Cell Viability Assay, respectively.

2.3. MTT Cell viability assay

Growth of tumoral cells quantitated by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product. At the end of 72 hours incubation, the medium in each well was replaced by MTT solution (20 cell/well, 5 mg/ml in phosphate-buffered saline), the plates were incubated for 4 hours under 5% CO₂ and 95% air at 37°C. MTT reagent was removed and the formazan crystals produced by viable cells were dissolved in 100 DMSO and gently shaken. The absorbance was then determined by ELISA reader at 570 nm. The percentage growth inhibition was calculated using following formula,

$$\% \text{ cell inhibition} = 100 - [(At - Ab) / (Ac - Ab)] \times 100$$

Where, At = absorbance value of test compound, Ab = Absorbance value of blank and Ac = Absorbance value of control.

The effects of extracts were expressed by IC₅₀ values (the drug concentration reducing the absorbance of treated cells by 50% with respect to untreated cells).

3. STATISTICAL ANALYSIS

The data are expressed as mean \pm standard deviation (SD) for at least three independent determinations in triplicate for each experimental point. The data were analyzed using IBM SPSS Statistics 20 software. For all the measurements, Two-way ANOVA followed by Duncan's New Multiple Range Test ($P \leq 0.05$) was used to assess the statistical significance of difference between control and treatments.

4. RESULTS

To investigate the cytotoxic potential of extracts were prepared according to the traditional use in Iran from Iranian plants used in traditional medicine for the treatment of various diseases such as cancer, inflammation or infectious diseases. We collected the plant of *S. sahendica* in order to screen them for possible cytotoxic activity against human cervical cancer cell lines (HeLa) at different concentrations to determine the IC₅₀ (50% growth inhibition) by MTT assay.

4.1. Effects of ethanolic extract of *S. sahendica* on proliferation of HeLa cells

Results of different concentrations of *S. sahendica* including 0.156, 0.312, 0.625, 1.25, 2.5, 5, 7.5 and 10 mg/ml are tabulated in Table 1. MTT assay of *S. sahendica* shows significant effect on HeLa cell in concentration range between 0.156 mg/ml to 1.25 mg/ml compared with control. The highest cytotoxicity of this extract against HeLa cell was found in 0.625 and 0.156 mg/ml concentration with 68.02 and 66.16 percent of cell growth inhibition. It was found that the percentage of growth inhibition to be increasing with increasing concentration of test compounds, and IC₅₀ value of this assay was 3.45 mg/ml, thus *S. sahendica* extract at 0.156 to 10 mg/ml exhibited dose-dependent inhibitory effects on the proliferation of HeLa cells (Figure 1).

Table 1. Cytotoxicity activity of *S. sahendica* extracts against HeLa cell line at different concentrations by MTT assay

Concentrations of <i>S. sahendica</i> (mg/ml)	Absorbance	Inhibition (%)	IC ₅₀ (mg/ml)
0.156	0.308 \pm 0.248*	66.16	
0.312	0.360 \pm 0.288*	57.65	
0.625	0.300 \pm 0.214*	68.02	
1.25	0.355 \pm 0.225*	61.23	
2.5	0.541 \pm 0.249	33.66	3.45
5	0.635 \pm 0.250	21.14	
7.5	0.786 \pm 0.151	0.500	
10	0.787 \pm 0.100	0.100	
Control	0.800 \pm 0.098	-	
Monocyte	0.503 \pm 0.140	38.72	
DMSO	0.746 \pm 0.071	5.97	

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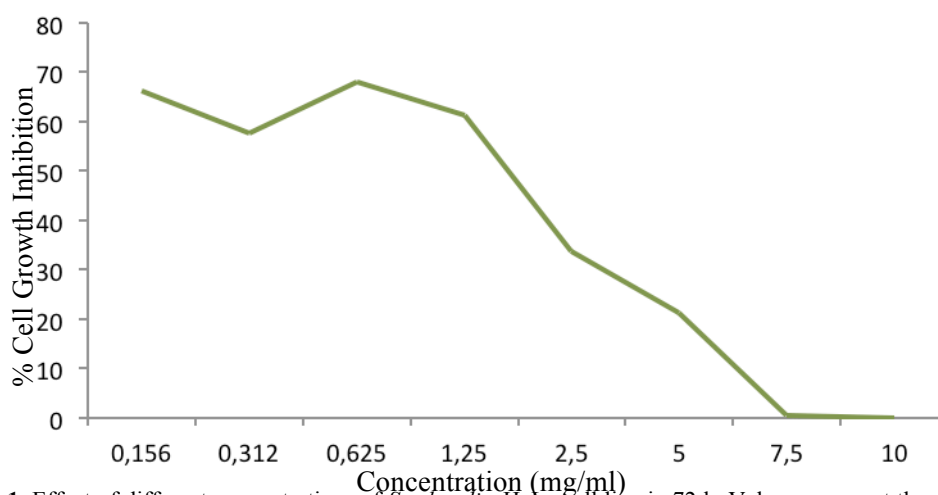


Figure 1. Effect of different concentrations of *S.sahendica* HeLa cell line in 72 h. Values represent the mean of three experiments.

Overall, this study evaluate that ethanolic extract of *S. sahendica* has potential cytotoxic activity on Hela cell, indicating the presence of cytotoxic compounds in these extracts. This study provides only basic data, further studies are necessary for isolation and identification of biologically active substances from these extracts.

5. DISCUSSION

On a whole, our goal was to determine whether the extracts of these plants exerted an inhibitory effect on cancer cell proliferation and caused cell death. The results of our studies suggest that ethanolic extract of *S. sahendica* possess the strongest cytotoxic effects on human cancer cell. In the present study, we observed that the extract of *S. sahendica* caused marked cell growth inhibition in the human cervical cancer HeLa cell line in a dose –dependent, but this effect is caused in the lower concentration. Studies have shown differential sensitivities to several natural compounds between tumor and normal cells in vitro, and the results obtained from the present study show that the ethanolic extract from *S. sahendica* is cytotoxic to HeLa cell lines. In this preliminary study, we have focused our interest on crude plant extracts, the cytotoxic activity could be due to the presence in the ethanolic extract of active products that could probably have highly anti-growth effects.

On the other hand, some studies revealed the presence of terpenoid, flavonoids and alkaloids in the extract of *S. sahendica*, which could be responsible for this activity (Esmaeili et al., 2009; Salehi et al., 2007; Shaerzadeh et al., 2011a). Flavonoids have been found to possess antimutagenic and antimalignant effects (Mosmann, 1983; Brown, 1980). Moreover it has protective effect against cancer by their effect on signal transduction in cell proliferation and angiogenesis. Several studies have been reported on the phytochemical and other biological properties of *S. sahendica* (Salehi et al., 2007; Shaerzadeh et al., 2011b). For example Esmaeili et al. (2009) reported that *S. sahendica* has potent hepato-protective effect against alcohol induced tissues damage in experimental animals. This study also suggests that possible mechanism of this activity may be due to the presence of flavonoids and phenolics compound(s) in the methanolic extract of *S. sahendica* which may be responsible to hepato-protective activity. They showed methanolic extract of *S. sahendica* had a potent increasing effect on GSH level and vitamin C and E contents on liver and kidney tissues compared to alcohol treated rats.

Also, Shaerzadeh et al. (2011b) investigated the protective effect of *S.sahendica* against H₂O₂-induced cell death in rat pheochromocytoma (PC12) cells. their results indicate that *S.*

sahendica protects PC12 cells treated with H₂O₂ via suppression of upstream factors of apoptosis pathway.

Therefore, this plant could be as a source for new lead structures in drug design to combat cancer. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent against cancer and we therefore, suggest further, the purification and characterization of the phytochemicals and isolation the active constituent. In addition, it can be subjected to pharmacological screening of this plant along with investigations that are needed to provide some additional insight into the *in vivo* cytotoxic activity of the plants with a view to obtaining useful chemotherapeutic agent.

Thus, further studies are required to elucidate the precise molecular mechanisms and targets for cell growth inhibition which will allow the rationale design for more effective molecules for the eventual use as cancer chemopreventive and/or therapeutic agents.

The current study has demonstrated that ethanolic extract of a commonly used in Persian medicinal herb, *S.sahendica* in its natural form, could significantly suppress the proliferation of cervical cell line (HeLa) *in vitro* by means of the MTT assay. Based on the results of this experiment extract of *S. sahendica* has inhibitory effect on HeLa cell line, and could be useful in variety fields as the anticancer complex.

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