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The anticancer effects of *Stachyslavandulifolia* extract

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Abstract

Stachys is one of the largest genera in the flowering plant family. It is in the subfamily Lamioideae (Labiatae), spreading and growing in different parts of Iran. The purpose of this study was to investigate the chemical properties of *Stachyslavandulifolia* as well as the essence, ethanol and methanol extracts of this plant. It was collected from Chulus located in the West of Mazandaran Province. The ethanol and methanol extracts were provided using soxhlet and percolation. The extracts then were defatted and solvent removal was performed in a subsequent process. The anticancer activity of extracts was also conducted using MTT assay method in this respect. The test results indicated that there is no significant effect on the inhibition of cell growth when the concentration of extracts is lower than 0.5 mg/mL clarifying that the more the increase in concentration, the more the reduction in growth inhibition.

Keywords: *Stachyslavandulifolia*; extract, soxhlet; anticancer; labiatae.

Introduction

Flowers and plants are silent presences; they nourish every sense except the ear [6], they produce no sound but they are so expressive, as they are a glorious manifestation of the power and majesty of God.

Since the medicinal herbs are highly effective nutritional supplement and of

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particular importance in treating diseases, it has been interested by researchers to identify the compositions of such plants namely the medicinal and aromatic species, especially those native to the land [2]. The *Stachys*, with over 270 species, are widely used in traditional medicine in Iran and has the honor of being one of the largest families of Labiatae around the world, especially in Mediterranean area with over 4000 species and 200 genera [7]. Iran is rich in species of these plants that grow as wild plants of the approximately 3000 species of medicinal herbs known to occur in the wildlife world [6] among which 140 species are native or have occurred in Iran [1]. Cancer is a dynamic process, caused by unknown elements and multiple independent variables that can lead to cellular and molecular changes.

As we know, cancer is a condition wherein cells grow in a specific part of the body and reproduce uncontrollably. The cancerous cells can invade and destroy surrounding healthy tissue, including organs. In traditional medicine, cancer is categorized under "swellings" and classified as "solid tumor" and "cold swelling" [4].

Statistics, today, reveal for the first time that cancer is the biggest cause of death worldwide with 8.2 million people dying per year. Early detection of cancer can greatly improve the odds of successful treatment and survival, however, it is worthy to be mentioned that the adjuvant treatments and therapies are frequently associated with adverse side effects. Considering less favorable response to treatment, it is necessary to try to develop more effective, less toxic drugs in this respect and plants are cases in point. They are used in traditional medicine to treat cancer [3]. Many countries today are seeking to find plants and derived compounds that can be useful in treatment or can be used as natural materials in different applications. Although many studies on species and important compounds have appeared in recent years, there are still many areas to be touched and explored [2]. Many attempts have been made by Iran research center and all the major research centers in the world to develop effective drugs and medicines that can be selective in killing cancer cells while having little effect on healthy cells and medicinal herbs are considered to be a great source of developing new drugs in this regard [2]. It is accordingly the purpose of this article to examine and study the *Stachyslavandulifolia* as one of the medicinal herbs.

Experimental

Plant materials: All parts of the plant *Stachy-*

slavandulifolia were collected from Chalus (North of Iran) in the spring of 2012. The samples were identified by Dr Abbas Ali Dehpour.

Preparation of ethanolic and methanolic extract

Plants were dried at room temperature and all parts were extracted, then the percolation and the resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained(10.8%), which was then freeze-dried for complete solvent removal.

The anticancer effects of *Stachyslavandulifolia* and its detection method

Cell line:

Preparing from Pasteur Institute cell bank of Iran, the Hela cell line was used in this study and grown in RPMI 1640 Medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin and streptomycin.

Cytotoxicity test:

A cytotoxicity test determines whether a product or compound will have any toxic effect due to leaching on living cells. It is generally used as a screening tool for raw materials or component products before they are put into the design of a medical device, knowing that the cytotoxicity of ethanol and methanol extracts of aforementioned plant was determined by MTT-colorimetric test. The active mitochondrial dehydrogenises cleavage and reduce the soluble yellow MTT namely 5,2-3diphenyltetrazolium bromide salt (MTT) assay dyed into the insoluble purple formazan. The absorbance of this compound was with ELISA after dissolving in DMSO.

MTT Preparation manual:

In order to prepare methyl thiazolyltetrazolium solution, MTT from American Sigma Company, 25mg MTT powder was dissolved in 50 mL deionized water and sterilized by filtration.The resulting solution was kept in aluminum container at 4 °C; a screw on cap helped maintain a sterile pouring surface.

Studying the cytotoxicity of *Stachys* extracts using MTT test:

The Hela cell line as described before was grown in RPMI 1640 Medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1000 u/mL penicillin, 1000 u/mL streptomycin. They then were stored in sterile flasks, from Falcon company, at 37 °C and in an atmosphere containing 5% Co_2. After three days when the cells approximately covered all parts of the flask, the adhesive layer on the bottom of flask were isolated enzymatically using trypsin-versenesolution.

The cell suspension, after a defined time, was added to falcon tube containing the culture medium and then centrifuged. The growth medium on the cells was discarded and new medium was added to the culture flask and the hemocytometer was used to count the number of cells. The cell suspension was prepared and poured (1000 mL) in to a 96-well cell-culture plates using8-channel sampler. A row of wells containing no cells but culture medium was considered as a blank group and another one containing both cells and culture medium as a control group.

The culture plates were placed for 18 to 24 hours in a modular incubator chamber so that the cells can be relieved from stress induced by trypsin and returned to their normal condition. The compound under study was then prepared by appropriate dilution and (100 mL) was added into the well-cell plates in rows. The cells were placed for 72 hours in a modular incubator chamber at 37 °C and in an atmospheric pressure of 0.5 atm. 20 mL of the MTT solution was added to each well.

The plates then were incubated for 2-4 hours and the medium on the cells was discarded. 100 mL of dimethyl sulfoxide (DMSO) solvent was added to each well to dissolve MTT formazan. The plates were placed in shaker and the absorbance was read at 570 nm with a microplate reader (DynexMMx) in order to calculate the cell viability.

Results and discussion

The results of studying the anti-cancer effects of *stachyslavandulifolia* extract are as follows:

Statistical analysis:

The results were based on SEM or standard error of the mean. The difference between the values was determined by student test. There was a significant mean difference where $\boldsymbol{p < 0.05}$. It is worthy to be mentioned that all statistical analyses were conducted with Excel software.

Studying the results of cytotoxicity of*Stachys* extracts on Hela cell line:

The results of cytotoxicity of ethanol and methanol extracts on Hela cell line can be seen in Table 1 and 2 respectively. Each extract concentration was tested in three separate experiments (in triplicate). Hence, the values listed in table are the average percentage of responses to each of the three experiments considering the cell growth inhibition.

Table 1. The cytotoxicity of ethanol extract of Stachys on cell line (Hela) in different concentrations

Extract(mg/milt)	Absorb	Inhibition%
0.03125	0.370±0.14	-2.3
0.0625	0.14±1.290	11.50
0.125	0.11±1.260	15
0.25	0.08±1.202	23
0.5	*0.05±1.090	42.20
1	0.05±1.145	32
2.5	0.05±1.268	9.50
5	0.1±1.515	-33
7.5	0.2±1.775	-70
10	0.1±1.890	-96
Control	0.1±0.715	-98

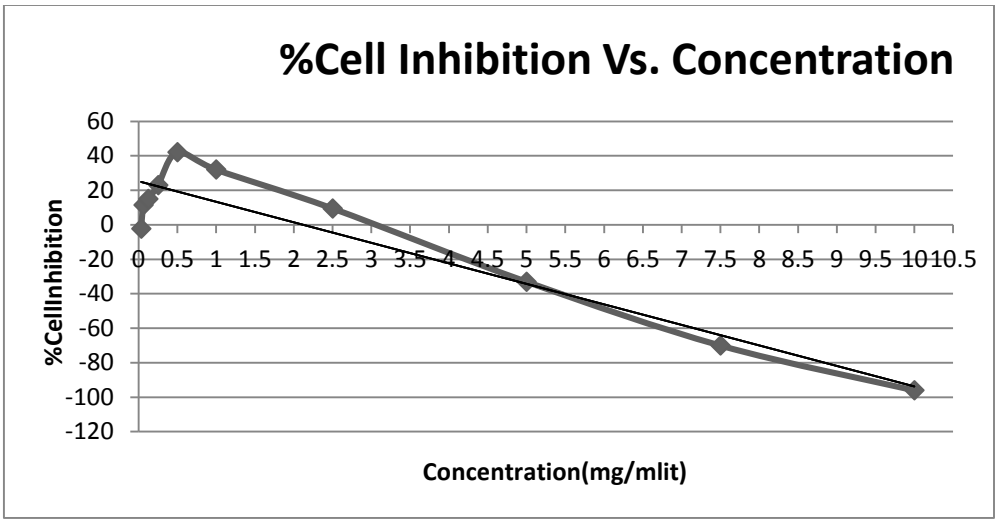

Figure 1. The cytotoxicity of ethanol extract of *Stachys* on cell line, using MTT test

Table 2. The cytotoxicity of methanol extract of *Stachys* on cell line (Hela) in different concentrations

Extract(mg/milt)	Absorb	Inhibition%
0.03125	0.14±1.374	-2.6
0.0625	0.14±1.297	12
0.125	0.11±1.263	15.33
0.25	0.08±1.210	23
0.5	❋0.05±1.093	42.66
1	0.05±1.149	32.33
2.5	0.05±1.271	10
5	0.1±1.529	-33.33
7.5	0.2±1.782	-71
10	0.1±1.899	-97
Control	0.715±0.1	-99

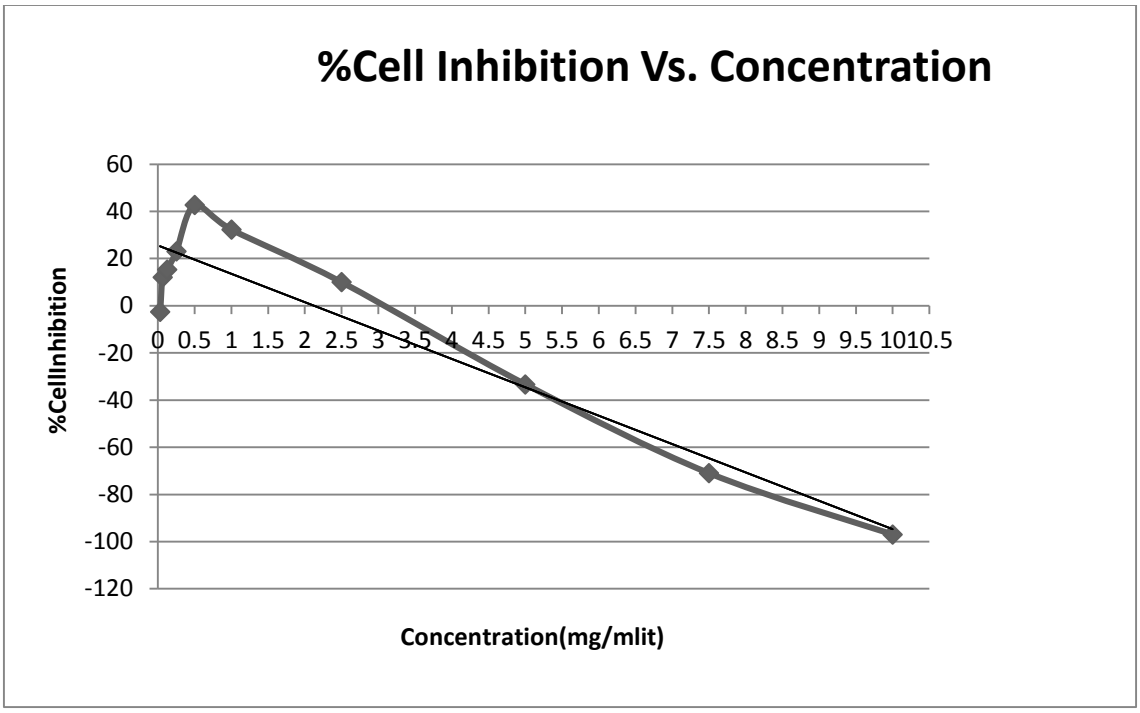

Figure 2. The cytotoxicity of methanol extract of *Stachys* on cell line, using MTT test

Anticancer effects of extracts

Many claims have been made regarding the effectiveness of medicinal herbs and their positive effects on cancer care and treatment; however, not many of these have been based on sound scientific evidence. Nevertheless, the discovery and development of anticancer agents could provide convincing evidence to confirm the role of plants as a source of new anticancer agents [4]. To find an anticancer herbal compound, being strong, safe and harmless, the cytotoxic effect of methanolic and ethanolic extracts of *Stachys*growing in the northern part of Iran was studied on Hela cancer cells. After 72 hours of treatment with methanolic and ethanolic extracts of *Stachys*on cancer cells, the results[3] showed that these extracts have no or very little effect on cancer development. The research results showed a significant reduction in cell growth, compared to that of control group, when using the concentration of 0.5 mg/mL. It is worthy to be mentioned that the *Stachys* showed no significant growth inhibition at concentration below 0.5 mg/mL clarifying that the more the concentration, the less the growth inhibition. The extracts can also be used for pharmacologic purposes in the defined concentrations.

Cancer is the biggest cause of death worldwide. There are some treatment options, but few of them are effective at

producing successful results or are frequently associated with adverse side effects [4]. Considering less favorable response to treatment, it is necessary to try to develop more effective, less toxic drugs in this respect and as mentioned before plants are cases in point.

Conclusion

As the studies showed, regarding the anticancer activity, the more the concentration of extract, the lower the cytotoxicity. It clarifies that there is no significant growth inhibition at concentration below 0.5 mg/mL; namely, the more the concentration of extract, the lower the amount of cell growth inhibition. Having the anticancer effect to some extent, the *stachys* extracts can be used for pharmacologic purposes in the defined concentrations. Considering less favorable response to treatment, it is necessary to continue the research to develop more effective, less toxic drugs in this respect.

In general speaking, the cytotoxic effects of the mint family of herbs are attributable to terpenoid compounds. Taking into account all these factors mentioned above, we can safely arrive at the conclusion that the terpenoids can be at least one of the factors that give rise to the formation of cytotoxic effects of *Stachys* as well.

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