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The Cytotoxic Activity of Various Herbals against Different Tumor Cells: An in Vitro Study

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Abstract

Background: Medicinal plants have been investigated for possible anti-cancer effects. The aim of the present study was to examine the cytotoxic activity of several medicinal native plants on different tumor cell lines.

Methods: Plants including *Salvia santolinifolia*, *Salvia eremophil*, *Salvia macrosiphon*, *Salvia reuterana*, *Teucrium persicum*, *Anvillea gracini* and *Francoeuria undulate* were collected from different sites of Fars Province in southern Iran. The methanolic extracts of the plants were prepared and their effects at concentrations of 5-200 µg/ml on various tumor cell lines were examined using a colorimetric assay.

Results: Among the extracts of *Salvia* species, the strongest inhibitory effect was observed for *S. reuterana* extract. This extract showed a strong cytotoxic effect on the Raji lymphoma cell line. More than 50% of Raji cells growth was inhibited by 21 µg/ml of this extract. *S. macrosiphon* extract also showed a strong inhibitory effect on this tumor cell line (IC50=77±1 µg/ml). The greatest inhibitory effect of *T. persicum* extract was on Hela tumor cell line. This extract at concentration of 69±2 µg/ml causes 50% inhibition of Hela cell growth. Corresponding data for *A. gracini* extract was obtained at concentrations of 83.5±2 and 86±3 µg/ml on Jurkat and Hela cells, respectively. *F. undulata* reduced the proliferation of all the tumor cell lines used in this study but this inhibition did not reach 50%.

Conclusion: All the extracts, more and less, showed inhibitory effects on the tumor cell lines. The most cytotoxic activity was observed in *S. reuterana* with an IC50 value less than 25 µg/ml towards Raji cell line.

Keywords: *Salvia santolinifolia*; *Salvia eremophil*; *Salvia macrosiphon*; *Salvia reuterana*; *Teucrium persicum*; *Anvillea gracini*; *Francoeuria undulat*

Introduction

Medicinal plants have been regarded as important sources that could produce potential chemotherapeutic agents for cancer treatment.[1-4] Based on cytotoxicity bioassay, over 400 compounds have been isolated from plants.[5] Vinblastin, vincristine, etoposide and taxol are prominent examples of plant-derived compounds accredited as anti-cancer drugs. Most of these chemotherapy drugs exert their anti-cancer effects by arresting cells at different stages of the cell cycle and then promoting them into apoptotic cell death.[6-8] The majority of these agents have only a limited anti-solid tumor activity and generate many side effects.[9] Therefore, developing new anti-cancer drugs with a higher potency and specificity against cancer cells has become an important goal in biomedical research.

Human cell lines have been used extensively to study the anti-tumor activity of medicinal plants. In the present study using various cell lines, we investigated the cytotoxic activity of several medicinal herbals that are native to Iran. These plants were either traditionally used in treating cancer or were not studied before. However, they belong to a genus with some species which have been shown to have anti-tumor activity. They include *Salvia santolinifolia Boiss*, *Salvia eremophil Boiss*, *Salvia macrosiphon Boiss*, *Salvia reuterana Boiss* and *Teucrium persicum Boiss* that belong to *Labiatae* (Na-na in Persian)

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family and *Anvillea gracini* (Burm) and *Francoeuria undulate* (*L.*) that belong to *Compositae* (Kasni in Persian) family.

The genus *Salvia* consists of a group of plants containing at least 900 species found in temperate and subtropical regions throughout the world.[10] Fifty eight species of *Salvia* are native and distributed around Iran. The antimicrobial and anti-tumor activity of some *Salvia* species has been reported. In traditional medicine, *S. miltirrhizae*, an important species of this genus has been used for treatment of cancer for decades in South East Asia.[11] Four species of this genus were included in our study. Among them, *S. macrosiphon* is used in folk medicine as antiseptic, anti-inflammatory and spasmolytic.[12] Three other species have been used for treatment of infections.

The genus *Teucrium* comprising over 300 plant species is endemic to the Mediterranean region and the Middle East. The derivatives from several species are dispensed for the treatment of obesity, hypercholesterolemia, and diabetes, as well as for their anti-inflammatory, anti-microbial and anti-cancer properties.[13] *T. persicum* is the endemic plant of Iran and is distributed only in the south of Iran.

The genus *Francoeuria* belongs to the family Compositae that is one of the largest groups of flowering plants with about 1100 currently accepted genera and 25000 species.[14] Some species of *Francoeuria* are traditionally used for the treatment of rheumatism, arthritis, gout and other forms of inflammation.[15] *F. undulate* is the only species that grows in Iran.

Anvillea species are used in the folk medicine as excellent heating, for the treatment of dysentery, gastrointestinal disorders and has been reported to have hypoglycemic and anti-tumor activity.[16] Among the plants in the genus *Anvillea* (*Compositae–Inuleae–Inulinae*), *A. gracini* is found in Iran and is distributed in the south east of the country.

Materials and Methods

Samples of fresh aerial parts of the plants were collected in the spring from Fars Province in the south of Iran. The plants were identified by Dr. Khosravi from the Biology Department, Shiraz University, Shiraz, Iran. Voucher specimens were deposited at the Herbarium of the Shiraz University. The methanolic extract of the plants at concentrations of 5-200 µg/ml was used and IC50 (concentration of plant extract at which half the cell growth is inhibited) value was determined. Doxorubicin was used as a reference compound.

After washing and drying, the aerial parts were powdered and defatted with petroleum ether for 4 hours. Methanol extract was obtained by maceration of the plants in methanol at room temperature for 48 hours. The methanol extract was filtered and concentrated under reduced pressure. The yield (w/w) of the extracts was 11.6% *S. eremophila*, 10% *S. macrosiphon*, 11.1% *S. reuterana*, 10.3% *S. santolonifolia*, 13.4% *T. persicum*, 12.8% *A. gracini* and 12% for *F. undulata*. The dried extracts were later dissolved in DMSO followed by RPMI medium to obtain 2 mg/ml solution. The solution was filtered for sterilization and then diluted with the medium to prepare appropriate concentrations.

Tumor cell lines that were used in this study included Hela (Cervix epitheloid carcinoma), Raji (Burkitt's lymphoma), Fen (bladder carcinoma), K562 (myelogenous leukemia) and Jurkat (T cell leukemia) prepared from Iranian cell bank, Tehran, Iran. All the cell lines were kept in RPMI 1640 medium (Sigma, St, Louis, USA) supplemented with 10% fetal calf serum (Gibco, Germany) in culture flasks at 37°C in 5% humidified CO_2 incubator. The cells were fed until confluence, being expanded by trypsinization (for adherent cells), and sub-cultured at lower numbers in new culture flasks. Viability of the cells was determined by trypan blue dye exclusion test.

A colorimetric assay using 3 (4, 5-dimethylthiazoyl-2, 5-diphenyltetrazolium bromide (MTT) (Sigma) was performed. Briefly, the cells were added onto the flat-bottomed micro-culture plates in the presence or absence of the various concentrations of the extracts (in triplicate) and incubated at 37°C in a 5% humidified CO_2 incubator for 48 hours. Then, 10 µl of MTT (5 mg/ml) was added to each well and incubation was continued for a further 4 hours at 37°C. 100 µl/well of solubilization solution containing isopropanol and 10% SDS in 0.01 M HCL was added into each well. After complete solubilization of the dye, the plates were read at 570 nm on an ELISA reader (Pharmacia, Sweden). The reference wavelength was 690 nm. The mean optical density (OD ±SD for each group of replicates was calculated. The percentage of inhibition of cells exposed to the various treatments was obtained as follows: Inhibition%=100-[(Test OD / Non-treated OD) x 100]. Non-treated cultures in all the experiments contained the solvent but not the extract.

Results

As shown in Figure 1, all *Salvia* species showed some inhibitory effects on all the cell lines. Among the extracts of *Salvia* species, the weakest effect belonged to *S. santolinifolia*. The inhibitory effect of this extract at the highest concentration on the tumor cell lines did not exceed 41.5%. *S. eremophil* also showed a weak acivity against different cell lines. The highest activity of this plant was against Fen bladder carcinoma with 46.6% growth inhibition at 200 µg/ml of the extract. The strongest inhibitory effect of *Salvia*

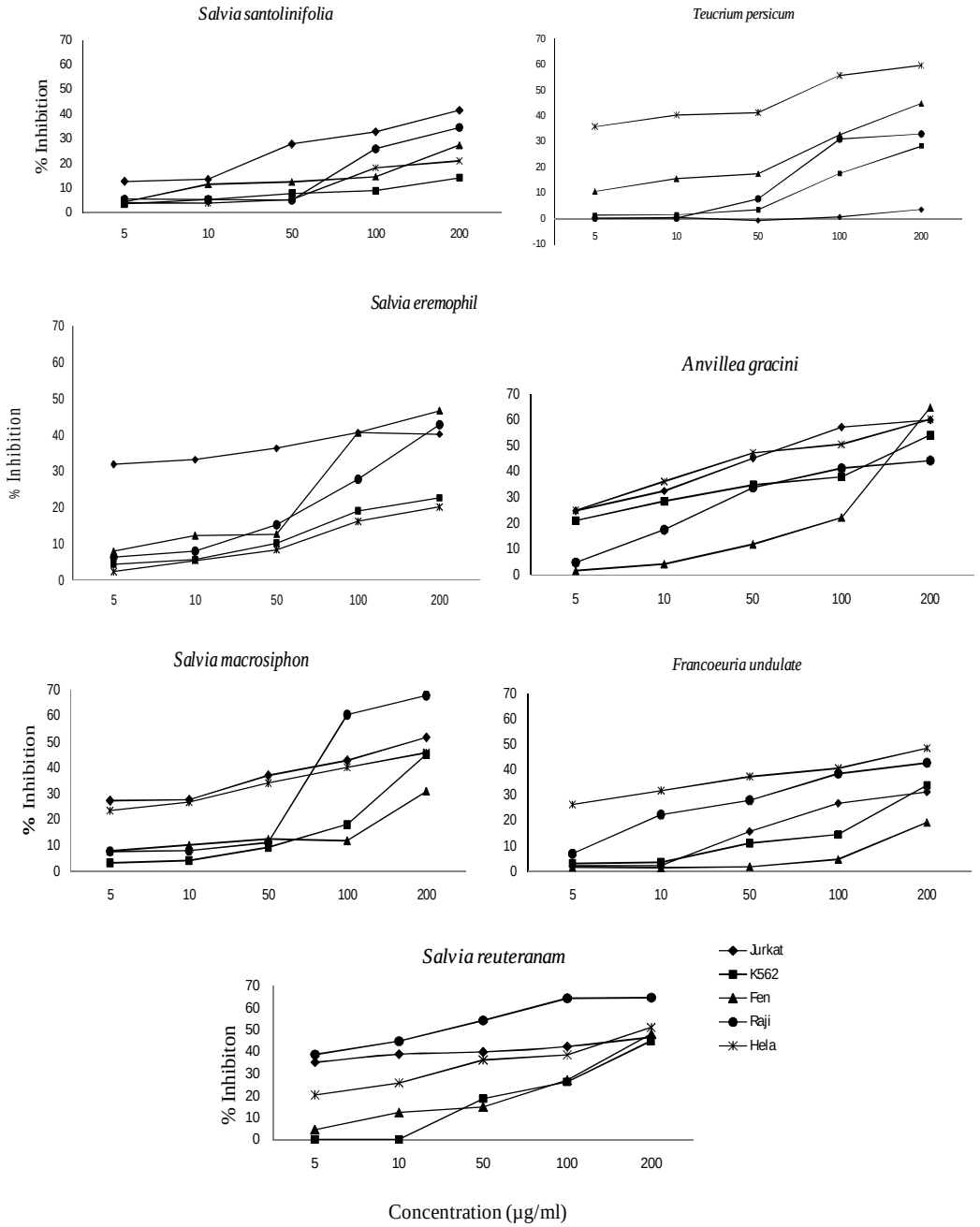

Fig. 1: Cytotoxic Effects of the methanolic extracts of several medicinal plants on different tumor cell lines.

Table 1: Cytotoxic activity (IC50 µg/ml) of the methanolic extract of various medicinal plants against different tumor cell lines

Extracts	Jurkat	K562	Raji	Fen	Hela
Salvia reuterana	158±6	>200	21±0.8	>200	156±5
Salvia eremoplila	>200	>200	>200	178±4	>200
Salvia macrosiphon	143±6	>200	77±1	>200	183±6
Salvia santolinifola	190±2	>200	>200	>200	>200
Tucrium persicum	>200	>200	>200	193±6	69±2
Anvillea gracini	83.5±2	168±4	181±3	157±5	86±3
Francoeuria undulate	>200	>200	>200	>200	167±4

species was observed for *S. reuterana* extract. Although the inhibitory effect of this extract on Jurkat, K562, Fen and Hela cells did not reach 50%, it showed a strong inhibitory effect on the Raji cell line. 50% of Raji cells growth was inhibited by 21±0.8 µg/ml of this extract (Table 1). *S. macrosiphon* extract also showed a strong inhibitory effect on Raji cells. Proliferation of more than 60% of these cells was inhibited by 100 µg/ml of the extract. The IC50 obtained for *S. macrosiphon* extract on Raji cells was 77± 1 µg/ml. The effect of the latter species on Raji cells was dose dependent and increased with addition of more extract to the culture.

A study of the effect of *T. persicum* on different cell lines showed its inhibitory effects on all cell lines except Jurkat that was resistant. Even at 200 µg/ml of the extract, only 3.6 % inhibition of proliferation in Jurkat cells was observed. Other leukemia cell lines including Raji and K562 were dose dependently inhibited by the extract. *T. persicum* at 200 µg/ml inhibited 44.8% of Fen cells. The greatest inhibitory effect of this extract was on Hela tumor cell line. The extract at concentration of 69±2µg/ml caused 50% inhibition of the growth of these cells.

A. gracini extract showed inhibitory effects on all the cell lines. The inhibitory effect of the highest concentration of the extract on Raji cells was 44.3% and on K562 it was 54.1%. Growth of Fen cells was also affected by this extract (IC50=157±5 µg/ml).The most inhibitory effect of *A. gracini* was on Jurkat and Hela cells with IC50 of 83.5± 2 and 86±3 µg/ml, respectively.

Addition of various concentrations of the *F. undulata* to different tumor cell lines weakly inhibited the proliferation of cells. Hela cells were more sensitive than others with IC50 of 167±4 µg/ml. The order of sensitivity of the cell lines to this extract at 200 µg/ml was Hela (48.6%), Raji (42.8%), K562 (33.9%), Jurkat (31.3%) and Fen (19.3%), respectively.

Discussion

In the present study, several plants native to Iran and belonging to Labiatae and Compositae family with a wide distribution to the country were investigated for possible anti-tumor effects on different cell lines. The tumor cell lines used in this study that were from leukemia and carcinoma cells origin responded to the extracts differently. The reason for this difference perhaps reflects the difference between the effective components present in the extracts and their mode of actions.

Among the plants studied, *Salvia* was expected to show more cytotoxic activity against the tumor cells. This was due to the presence of several reports showing the existence of various cytotoxic compounds in different *Salvia* species. Salvicine, salvinal and tanshinone are examples of such compounds.[17,18] This compound has been used for treatment of breast cancer.[19] The ability of *Salvia* species including *S. perfoliata*, *S. thymbra* and *S. officinalis* in inhibition of human tumor cell growth has also been shown.[20] In our previous study, *S. mirzayan* extract demonstrated an activity against proliferative lymphocyte in the growth inhibition assay.[21] As demonstrated in the present study, *Salvia* species all showed a dose dependent inhibition on the tumor cell lines particularly on Raji cells. This inhibitory effect was the strongest for *S. reuterana* and *S. macrosiphon*, respectively. These two plants showed an IC50 of almost 21 and 77 µg/ml against Raji cell line. This cell line is a Burkitt's lymphoma line originating from B cells. Stronger activity of the extracts of Salvia species against Raji cell may indi-

cate the sensitivity of tumors originated from this type of cells to Salvia species.

T. persicum is an endemic plant that has not been previously studied for its anti-tumor effects. As our study showed, Hela cell, which is a cell originating from epitheloid cervix carcinoma, was mostly affected by this extract. Weaker cytotoxic activity of *T. persicum* against other cell lines and stronger activity against Hela cells demonstrated the dependency of the effect of the extract on the origin and nature of the tumor cell lines. In a previous study, the anti-tumor activity of Teucrium diterpenoids against P 388 lymphocytic leukemia in mice has been reported.[22] *T. marum* has also been shown to have cytotoxic properties.[23]

In this study from the Compositae family, *F. undulate* and *A. gracini* were investigated. In a phytochemical and biological screening of Saudi medicinal plants, a compound (2alpha-hydroxyalantolactone) isolated from the principle of *F. crispa* has shown anti-leukemic effects,[24] Al-Yahya *et al.* introduced S-Carvotanacetone as the major component of *F. crispa* oil with cytotoxic properties.[25] In terms of *F. undulate*, no anti-tumor activity has been reported so far. As our results showed addition of various concentrations of the *F. undulata* to different tumor cell lines weakly inhibited the proliferation of cells. Hela cells were more sensitive than other cell lines to this extract.

In terms of *A. garcinii*, some new compounds (germacranolides and cis-parthenolid-9-one) with anti-tumor activity have been isolated.[26,27] In our study, *A. gracini* showed an inhibitory effect on Jurkat and Hela cells proliferation. The in vitro cytotoxicity of *A. gracini* against these cell lines is further evidence that strengthens the possible anti-cancer usefulness of this plant.

In conclusion, the results of our study showed that all the extracts examined, had some cytotxic effects on different cell lines. Among the extracts, *S. reuterana* was the strongest and could be considered as a suitable candidate for further studies to find the effective anti-cancer components.

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Conflict of interest: None declared.

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