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In vitro Cytotoxic Activity of Four Plants Used in Persian Traditional Medicine

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ABSTRACT

Purpose: The aim of this study was to investigate *in vitro* cytotoxic activity of four methanolic crude plant extracts against panel cell lines. ***Methods:*** Methanolic extracts were tested for their possible antitumor activity and cytotoxicity using the 3-(4,5-dimetylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay on six cancer cell lines; non-Hodgkin's B-cell lymphoma (Raji), human leukemic monocyte lymphoma (U937), human acute myelocytic leukemia (KG-1A), human breast carcinoma (MCF-7 cells), human Prostate Cancer (PC3) and mouse fibrosarcoma (WEHI-164) cell lines and one normal cell line; Human Umbilical Vein Endothelial Cells (HUVEC). ***Results:*** All species showed dose dependent inhibition of cell proliferation. IC50 values ranging from 25.66±1.2 to 205.11±1.3 µg/ml. The highest cytotoxic activity *Chelidonium majus L> Ferulago Angulata DC> Echinophora platyloba DC> Salvia officinalis L*, respectively. ***Conclusion:*** all extracts demonstrate promising cytotoxicity activity as a natural resource for future bio-guided fractionation and isolation of potential antitumor agents.

Introduction

Traditional medicine as an alternative therapy used for maintaining heath, boosting immune system function, prevention, therapy and remission of cancer. Nowadays; natural product can serve as chemotherapeutic agent and pharmaceutical application. In Iran, the use of traditional medicine is widespread practice.[1,2] The genus *Ferulago* from Apiaceae family are used in folk medicine for their sedative, tonic, digestive and anti-parasitic effects. Previous studies on the extracts compounds of *Ferulago angulata* revealed that contain ferulagone, β-hydroxy-13-epi-manoyl oxide, α-pinene, 2, 5-dimethoxy-p-cymene, p-cymene, and methyl carvacrol. Some of these compounds have both antibacterial and antifungal activities.[3] *Salvia officinalis* from Labiatae family is one of the commonly used in Iranian medicinal herb. Variety of pharmacological effects of *Salvia officinalis* extracts include antioxidant, anti-inflammatory, hypoglycemic and anti-mutagenic activities. *Salvia officinalis* contains tannic acid, oleic acid, ursonic acid, ursolic acid, cornsole, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, niacin, nicotinamide, flavones, flavonoid glycosides, and estrogenic substances.[4] *Echinophora platyloba* DC genus of Apiaceae is widely used in western and central part of Iran as a food seasoning and edible vegetable as an antifungal and antimicrobial preservative. *Echinophora platyloba* DC contains Coumarins, polyacetylenes, flavonoids, sesquiterpenes, and phthalides.[5,6] *Chelidonium majus* L. or the greater celandine from Papaveraceae family grows in North of Iran. Moreover, *Chelidonium majus* has been used in folk medicine as diuretic, choleretic and hypnotic. The most effective alkaloid components of the plant (chelidonine, chelerythrine, coptisine, sanguinarine, and berberine) have spasmolytic, antiulcer, anti-inflammatory, antimicrobial, antiviral, antifungal and antitumor activities and cytotoxic properties.[7,8]
These preliminary studies were evaluated for supporting cytotoxic activity of four species belonging to native medicinal plant of Iran on panel cancer cell lines including Raji, U937, KG-1A, MCF-7, PC3, and WEHI-164 cell lines as a part of research for new bioactive compounds with biological activity form.

Materials and Methods

Preparation of Crude Extracts

The aerial parts of the plants were separated, shade dried and grinded into powder using mortar and pestle. Fifty grams of each species were extracted separately with methanol in soxholet apparatus. The extract solutions were filtered and concentrated in vacuum to obtain the crude methanolic extracts.

Cell Lines and Culture

The following cancer cell lines were used for this study: non-Hodgkin's B-cell lymphoma (Raji), human leukemic monocyte lymphoma (U937), human acute

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myelocytic leukemia (KG-1A), human breast carcinoma (MCF-7 cells), human Prostate Cancer (PC3), mouse fibrosarcoma (WEHI-164) cell lines and Human Umbilical Vein Endothelial Cells (HUVEC) were obtained from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Cells were cultured into 75cm2 flasks containing RPMI- 1640 medium supplemented with Fetal Bovine Serum (FBS,10%, v/v) (Sigma, Germany), 100 U/ml penicillin and 100 µg/ml streptomycin (Sigma, Germany) in 5% CO2 at 37 °C.[9]

Cytotoxic Assay

Cytotoxic assay was performed using MTT reagent (3-(4, 5-dimetylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide) (Sigma, Germany) according to the manufacturer's protocol. Viable cells (1.5×10^4) from each cell line were seeded in 96-well flat bottom plates. When cells reached more than 80% confluence, the medium was replaced and cells were incubated with crude extracts at 0, 50, 100, 200, 300, 400, 600 µg/ml concentrations, at a maximum concentration of dimethyl sulphoxide (DMSO) 0.05% (v/v). A chemotherapeutic anti-tumor drug, Taxol at a final concentration of 20 µg/ml was added as the positive control. After 24h, the supernatants were removed and cell layers were washed with phosphate buffer saline (PBS, Invitrogen Gibco) and incubated with MTT (50 µl, 2 mg/ml) in RPMI 1640 for 4 h in a humidified atmosphere at 37 °C. The cell cultures were centrifuged at 1000 g for 5 min and the supernatants were discarded. Subsequently, 200 µl of dimethyl sulfoxide (DMSO, Sigma, USA) and 25 µl Sorenson buffer were added to dissolve the formazan crystals formed.[10] The optical density (OD) colored solution was quantified at 570 nm wavelengths by an enzyme linked immunoabsorbent assay reader (ELISA Reader, Bio-Rad). The absorbance of untreated cells was considered as 100%. Each extract and control was assayed in triplicate in three independent experiments. Fifty percent of inhibition concentration (IC50) was calculated by Graph Pad Prim 4 software. Percent growth inhibition of cells exposed to treatments was calculated as follows: % Inhibition = 100 - (Test OD/Non-treated OD) × 100). Concentration that inhibits 50% of cell growth was used as a parameter for cytotoxicity.[11]

Statistical Analysis

The data are expressed as mean ± standard deviation (SD) for at least three independent determinations in triplicate for each experimental point. The percentages of cell growth were used to obtain the full dose response curves and to determine the IC50 values (concentration inhibiting of 50% the cell growth compared with control).

Results

In the present study, the cytotoxic effect of 4 methanolic plant extracts on six cancer cell lines (Raji, U937, KG-1A, MCF-7, PC3, and WEHI-164) and one normal cell line **(**HUVEC) was determined using the MTT assay at a range of 0-600 µg/ml after 24 h of treatment. The *in vitro* cytotoxic activities of each plant extract are shown in Table 1 and IC50% values were determined from these dose response curves.

Comparison the crude extracts exhibit highest significant cytotoxic activity of *Chelidonium majus L*, against all tumor cell lines with lower IC50% values. Moreover, *Ferulago Angulata DC, Echinophora platyloba DC, Salvia officinalis L*, showed tumor selective cytotoxic activity depend on the cell line type. WEHI-164 was the most sensitive cell line and U937 was the most resistant tumor cell line against crude extracts treatment. None of the extract assayed exhibited significant cytotoxicity against HUVEC cell line.

Table 1. *In vitro* growth inhibitory activity (IC50 µg/ml) of four crude methanolic extracts.

Species	Cell lines IC50 (µg/ml)±S.D						
	Raji	U937	KG-1A	MCF-7	PC3	WEHI-164	HUVEC
Salvia officinalis L.	166.89±2.2	205.11±1.3	179.00±3.2	142.43±1.3	75.78±2.8	39.95±1.1	>600
Ferulago Angulata DC.	97.21±2.7	158.00±1.0	182.44±4.3	121.59±1.6	116.72±2.0	31.92±0.9	>600
Echinophora platyloba DC.	125.94±1.7	178.42±0.7	140.39±2.8	154.12±2.2	69.38±3.2	40.77±2.7	>600
Chelidonium majus L.	73.74±1.1	138.66±2.4	81.92±0.9	95.38±2.6	55.63±0.5	25.66±1.2	>600

*Data presented are the mean ± SEM of three independent experiments. p<0.05

Discussion

The cytotoxic effect of the crude methanolic extract of *Chelidonium majus L, Ferulago Angulata DC, Echinophora platyloba DC,* and *Salvia officinalis L* were investigated *in vitro* using MTT assay. MTT assay measured the cell viability based on the reduction of yellow tetrazolium MTT to a purple formazan dye mitochondrial dehydrogenase enzyme. So, the amount of formazan produced reflected the number of metabolically active viable cells. MTT results showed that all extract possessed cytotoxic effect against non-Hodgkin's B-cell lymphoma (Raji), human leukemic monocyte lymphoma (U937), human acute myelocytic leukemia (KG-1A), human breast carcinoma (MCF-7

cells), human Prostate Cancer (PC3), and mouse fibrosarcoma (WEHI-164) cell lines in a dose-dependent manner. Such anti-proliferative activity of these extract were characterized by the dose-dependent and tumor-selective manner, as reflected by the comparatively low IC50 values and the absence of significant effects on Human Umbilical Vein Endothelial Cells (HUVEC).

Conclusion

Our studies demonstrate the *in vitro* cytotoxic activity of methanolic extract of *Chelidonium majus L*, *Ferulago Angulata DC*, *Echinophora platyloba DC*, and *Salvia officinalis L* on non-Hodgkin's B-cell lymphoma (Raji), human leukemic monocyte lymphoma (U937), human acute myelocytic leukemia (KG-1A), human breast carcinoma (MCF-7 cells), human Prostate Cancer (PC3), mouse fibrosarcoma (WEHI-164) cell lines and Human umbilical vein endothelial cells (HUVEC), thus possibly suggesting as a natural resource for future bio-guided fractionation and isolation of potential antitumor agents.

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Conflict of Interest

The authors report no conflicts of interest.

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