



Cytotoxic Activity of Selected Iranian Traditional Medicinal Plants on Colon, Colorectal and Breast Cancer Cell Lines

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ABSTRACT

Background: Many natural products from plants have been recognized to exert anticancer activity. In this study, ethanolic extracts of selected medicinal herbs from Iranian flora including *Alyssum homolocarpum* Fisch. (from seeds), *Urtica dioica* L. (from aerial parts), *Cichorium intybus* L. (from roots) and *Solanum nigrum* L. (from fruits), were evaluated for their cytotoxic effect on different cell lines.

Methods: Cytotoxic effect of these extracts was studied on three different cancer cell lines; colon carcinoma (HT-29), colorectal adenocarcinoma (Caco-2) and breast ductal carcinoma (T47D). In addition, Swiss mouse embryo fibroblasts (NIH 3T3) were used as normal nonmalignant cells. MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was utilized for calculating the cytotoxicity of extracts on cell lines.

Results: Results showed the potent cytotoxic activity of *U. dioica* ethanolic extract against T47D cell line with IC₅₀ value of 46.14±4.55 µg/ml. Other extracts showed poor activity with IC₅₀>100 µg/ml.

Conclusions: Cytotoxic activity recorded in the present study revealed high potential antiproliferative activity of *U. dioica* ethanolic extract against T47D cell line. The real IC₅₀ values of this extract may be considerably lower than the IC₅₀ measured in our study if its pharmacological active compounds become pure. The results emphasize the importance of studies on *U. dioica* ethanolic extract to characterize potential components as cytotoxic natural medicines.

Introduction

Natural products, especially those of herbal and marine based were first introduced as important sources of anticancer agents in the 1950s by the US National Cancer Institute.¹ Among the patients with cancer in the USA, the use of complementary and alternative medicine, represented mainly by plants, ranges between 30-75%.² In this situation, ancient references of traditional medicine can

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increase the chances of finding anticancer agents from natural sources in comparison to random approaches.^{3,4} In this study, our aim was to investigate the cytotoxic effect of ethanolic extracts of selected medicinal herbs from Iranian flora including *Alyssum homolocarpum* Fisch. (from seeds), *Urtica dioica* L. (from aerial parts), *Cichorium intybus* L. (from roots) and *Solanum nigrum* L. (from fruits) on different cell lines.

Urtica dioica L. is used to treat rheumatic pain, colds, cough and liver insufficiency.⁵ Previous studies on *Urtica dioica* L. have shown that it has anti-inflammatory and anti-rheumatic, acute diuretic, natriuretic and hypotensive effects and stimulates proliferation of human lymphocytes.⁶⁻⁹ In addition, it has a powerful antioxidant activity against various oxidative systems in vitro.⁵ Compounds such as steroids, terpenoids, phenylpropanoids, lignans, coumarins, polysaccharides and lectins have been isolated from the roots of the plant.¹⁰

Solanum nigrum L. has been used to cure inflammation, edema, mastitis and hepatic cancer in traditional Chinese medicine.¹¹ Recent studies have shown that *Solanum nigrum* L. has anti-neoplastic effects on several human tumor cell lines.¹² Furthermore, it has also been indicated that an ethanol extract from ripe fruits of *Solanum nigrum* L. inhibited the proliferation of human MCF-7 breast cancer cells and induced cell death by apoptosis.¹³

Cichorium intybus L. is a potent anti-hepatotoxic plant. The alcoholic extract of *C. intybus* is used against pyorrhea or gingival inflammation. In addition, it has been demonstrated that aqueous and alcoholic extracts exhibited anti-inflammatory activity against formalin induced edema.¹⁴ *Alyssum homolocarpum* Fisch. has been used to cure dry cough, whooping cough, asthma, pneumonia and kidney stones in Iranian traditional medicine.¹⁵

The purpose of this study was to point out the cytotoxic activity of ethanolic extracts of *Solanum nigrum* L., *Alyssum homolocarpum* Fisch., *Urtica dioica* L. and *Cichorium intybus* L. against Caco-2

T47D, HT-29 and NIH-3T3 cell lines, using the MTT assay and based on their use in Iranian traditional medicine as anticancer medicinal plants and consider the possibility of utilization of the samples in the future for isolation of active compounds.¹⁶

Methods

Plant materials

Aerial parts of *U. dioica*, fruits of *S. nigrum*, roots of *C. intybus* and seeds of *A. homolocarpum* were purchased from herbal markets and authenticated by a taxonomist. Voucher specimens were deposited in the herbarium of BarijEssence pharmaceutical company, Kashan, Iran. After air drying, the plants samples were crushed by a mechanical grinder to optimum particle size.

Extraction

Each powder (100 g) was extracted with ethanol/water (80:20 v/v) for three successive 48 hours separately in percolator at room temperature. Obtained extracts were concentrated and dried with rotary evaporator.

Cell culture

RPMI 1640 cell culture medium was used to culture colon carcinoma (HT-29), colorectal adenocarcinoma (Caco-2) and breast ductal carcinoma (T47D) cell lines. For HT-29 cells, 10% fetal bovine serum (FBS) and for Caco-2 and T47D cells, 15% FBS was added to the medium. The Swiss mouse embryo fibroblast (NIH 3T3) cell line was kept in Dulbecco's modified Eagle's medium supplemented with 10% FBS. 100 IU/mL penicillin and 100 µg/mL streptomycin were added to the media. All the cell lines were incubated at 37°C and 5% CO₂ atmosphere.

Cytotoxicity assay

The ethanolic extracts were dissolved in dimethyl sulfoxide (DMSO) and further diluted with cell culture medium. Subsequently, different concentrations of each extract (50, 100, 250, 500 and 1000 µg/ml) were prepared. MTT assay was

Table 1. Yield of ethanolic extracts of selected plants and their cytotoxic activity (IC₅₀ values) against different cell lines.

Scientific name	Yield (%)	IC ₅₀ values of cell lines ¹ (µg/ml)			
		T47D	Caco-2	HT-29	NIH 3T3
<i>Alyssum homolocarpum</i> Fisch.	0.42	285.63 ± 44.42	386.43 ± 21.09	> 1000	311.9 ± 38.4
<i>Urtica dioica</i> L.	11.21	46.14 ± 4.55	> 1000	> 1000	186.06 ± 2.12
<i>Cichorium intybus</i> L.	20.04	443.01 ± 3.21	820 ± 23.11	> 1000	165.63 ± 9.73
<i>Solanum nigrum</i> L.	5.99	> 1000	579.21 ± 6.98	610.68 ± 123.24	401.55 ± 21.78



used to measure cytotoxic activity. 1×10^4 cells of each cell line were seeded into 96-well plates and incubated at 37°C. Different concentrations of each extract were replaced by media after 24 hours of incubation. After 72, 96, 96 and 120 hours of incubation for HT-29, T47D, NIH-3T3 and Caco-2 cells, respectively, 20 µl of MTT reagent (5 mg/ml) in Phosphate Buffered Saline (PBS) was added to each well and they were incubated in 37°C for 4 hours. After evacuation of the media, formed blue formazan crystals were dissolved in 100 µl of DMSO and incubated for 10 minutes at 37°C. Finally, the absorbance was measured at 570 nm using a micro plate reader (Anthos, Austria). All tests were repeated three times and cytotoxicity was considered as the median growth inhibitory concentration (IC50). According to WHO guidelines, further purification of crude herbal extracts is needed for extracts with IC50 values lower than 50 µg/ml after cytotoxic screening programs.

Statistical analysis

Data were presented as mean \pm SD of three independent experiments. IC50 (the median growth inhibitory concentration) values was calculated from dose-response curves using Sigma plot 10 software.

Results

In the present study, different concentrations of ethanolic extracts were added to Caco2, HT29, NIH 3T3 and T47D cell lines to examine their anti-proliferative effects. IC50 values obtained from MTT assay are shown in table 1. Our results showed that ethanolic extract of *U. dioica* has potent cytotoxic effect on T47D cell line with 46.14 ± 4.55 µg/ml IC50 value but other extracts do not have a significant cytotoxic effect on the cell lines.

Discussion

In this study, the cytotoxic activity of four ethanolic extracts against HT-29 (colon adenocarcinoma), T47D (breast ductal carcinoma), Caco-2 (colorectal adenocarcinoma) and NIH 3T3 (Swiss embryo fibroblast as normal cell line) cell lines was assessed by MTT assay. This assay is a well-established in vitro model for cytotoxicity evaluation against normal and cancerous cell lines.¹⁸

Different cytotoxic screening programs have adopted various IC50 values for further purification of crude herbal extracts. For example, WHO and US national cancer institute guidelines admit values lower than 50 µg/ml and 20 µg/ml, respectively.^{19,20} According to this information,

more purification is rational to investigate the active cytotoxic constituents in *U. dioica* ethanolic extract against T47D cell line based on its IC50 value. The cytotoxic potential of active plants is probably related to their different phytochemicals. Although previous studies have shown the potent cytotoxic activity of some glycoproteins and steroidal glycosides derived from *S. nigrum* against different cancerous cell lines including HT-29, MCF-7 (human breast cancer), PC-12 (human lung cancer) and HCT-116 (human colon cancer), its ethanolic extract from fruits in our study exhibit weak cytotoxicity against all the cell lines.²¹⁻²³

Traditional usage of *U. dioica* against cancer is not only limited to Iranian medicinal plants but also some reports have shown the wide application of *Urtica* species in Turkish traditional medicine to fight cancer. It has become a source of folk medicine for the treatment of many diseases. The leaves and roots are both used internally as a blood purifier and diuretic and an infusion of the plant is used for nasal and menstrual hemorrhage, diabetes, rheumatism, eczema, anemia, hair loss and as an expectorant and antidiarrheal. Compounds such as steroids, terpenoids, phenylpropanoids, lignans, coumarins, polysaccharides and lectins have been isolated from the roots of the plant.¹⁰ Previous studies have shown cytotoxic effects of aqueous extracts of aerial parts of *U. dioica* on KB (human epidermoid carcinoma), B16 (mouse melanoma), HeLa (human epithelial carcinoma) and HLA (human hepatoma) cell lines.^{24,25} Moreover, it had been shown that methanolic extract of roots of Nettle (*Urtica dioica*) can inhibit proliferation of epithelial prostate cancer cells due to its phenols and flavonoids like caffeic malic acid, caffeic acid, chlorogenic acid and quercetin.²⁶ Therefore, the cytotoxic activity of *U. dioica* against T47D cell line can be attributed to its immunomodulatory effect and phenolic phytochemicals. On the basis of the results of this study, the desirable cytotoxic effect of ethanolic extract of *U. dioica* (aerial parts) against T47D cell line was demonstrated. However, further purification is needed to find its principal active components.

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Conflicts of interest

The authors declare that they have no conflict of interest.



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