

Evaluation of the Cytotoxic Activity of Different *Artemisia Khorasanica* Samples on Cancer Cell Lines

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Summary

This study was the first to investigate the cytotoxic activity of *Artemisia khorasanica* samples from different places of Razavi khorasan province.

Three *A. khorasanica* samples were collected from different places of Iran. Cytotoxicity of their methanol, ethylacetate, dichloromethane and hexan extracts were evaluated on human gastric (AGS), cervica (Hela), colon (HT-29) and breast carcinoma (MCF-7) cell lines by quantitative MTT assay and their growth inhibitory activity was showed as IC₅₀.

All samples showed toxicity on cancer cells. Dichloromethan extract of sample 1 showed the most strong and all hexan extracts showed the weakest cytotoxicity. MCF-7 was the most sensitive cell line.

It was concluded that vegetation of cultivation of this plant in different conditions on various clima could affect its constituents. Also, *A. khorasanica* could be also considered as a promising chemotherapeutic agent in cancer treatment and experiments for isolation and elucidation of their anti-tumoral compounds is udergoing.

Keyword: *Artemisia khorasanica*, Cytotoxicity, AGS, Hela, HT-29, MCF-7, MTT assay.

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Introduction

Cancer causes significant morbidity and mortality and is a major public health problem worldwide (1). According to report of World Health Organization (WHO), approximately 80% of people in the world used traditional medicines to improving their primary health care needs (2). The most of cancer chemotherapeutants affect the normal cells, so the use of natural products for control of cancers has increased interesting (3,4). One approach is to study the anti-tumoral activity of plants based of prior informations about their usages in folk medicines (5,6).

The genus *Artemisia* L. (Astraceae, Compositae) is large and heterogenous, numbering over 400 species distributed mainly in the temperate zone of Europe, Asia and north America. (7-11). This genus has 30 species in Iran, among them *Artemisia khorasanica* is an endemic species of the genus that only found in Iran (11-13). Some classes of their chemical composition include monoterpenes, sesquiterpenes, sesquiterpene lactones, flavonoides, coumarins, sterols, polyacetylenes etc (9,14). Different species of *Artemisia* have a vast range of biological effects including antimalarial, antibacterial, antifungal (14) and antioxidant (16) activities. Available literature reports cytotoxic activity of different *Artemisia* species extracts on cancer cell lines *in vitro* (17-28).

As one step towards evaluating the anti-tumorigenic potential of different *Artemisia* species, methanol, ethylacetate, dichloromethane and hexan extracts of *A. khorasanica* collected from different places were prepared and treated with different human cancer cell lines. With the aim to identify the anti-proliferative principles present in *Artemisia khorasanica* from Iran, extracts were prepared. The human cancer cell lines were studied as models of tumors. This study designed to determined cytotoxic activity of

Materials and Methods

Plant material

Three samples from *Artemisia khorasanica* (aerial parts) collected from different regions of Razavi khorasan province (Table 1), (29). Dr. V. Mozaffarian, Research Institute of Forest and Rangelands, Ministry of Jihad Keshavarzi, Iran, was identified these plants. Voucher specimens of the species have been deposited in the Herbarium of School of Pharmacy, Mashhad University of Medical Sciences (MUMS), Mashhad, Razavi Khorasan province, Iran. The collected materials were dried under shade and stored in a cool place until analysis.

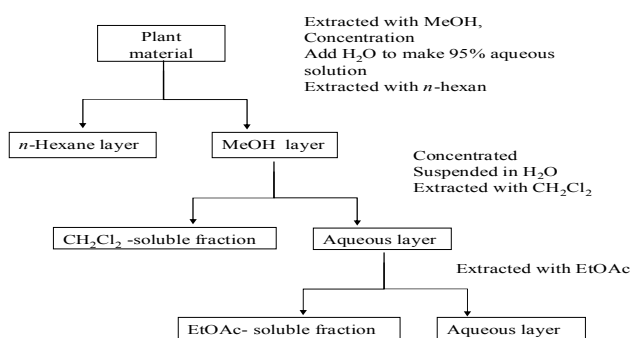
Table1. Characteristics of collected *Artemisia khorasanica* samples

<i>A. khorasanica</i> samples	Location	Collection time
Sample 1	Chovailly-Bajgiran road, Razavi Khorasan province, height 1780 m	2004/4/29
Sample 2	Cheshmeh gilās village, height 1155 m	2003/10/2
Sample 3	Near Sarakhs river, height 300 m	2003/8/14

Extraction Procedure

The shade dried aerial parts of each sample (100 g) were chopped in small pieces and then crushed into powder by a blinder. Each sample was macerated in pure methanol for 24 hours. The samples then extracted using a percolator. The extracted solutions were concentrated at 50 °C under reduced pressure to dryness. A small amount of water was added to MeOH to obtain a 95%-aqueous methanol solution. This solution was added to the concentrated extract was then extracted with an equal volume of n-hexane (3 times) to give a extract containing nonpolar compounds. The methanolic layer was evaporated to dryness and then suspended in water. The suspension was partitioned between CH₂Cl₂ and ETOAC, successively. Each obtained extract was concentrated at 50°C under reduced pressure to dryness (see figure 1). Finally water-soluble extract was concentrated by freez drying method.

Figure 1. Portioning scheme using immiscible solvent



Cell cultures and treatments

Four different cell lines, AGS (human gastric carcinoma cell line), Hela (human cervica carcinoma cell line), HT-29 (human colon carcinoma cell line) and MCF-7 (human breast carcinoma cell line) were obtained from Pasteure Institute Collection of Cell Cultures, Tehran, Iran. Cell lines were cultured as a monolayer culture in Eagle's medium, modified according to Dulbecco (DMEM from Gibco, USA) and supplemented with 10% fetal bovine serum (Gibco, USA), 1% penicillin/streptomycin (100 IU ml⁻¹ and 100 µgml⁻¹, respectively from Gibco, USA) in a 5% CO₂ humidified atmosphere at 37 °C. For the experiments, cells were removed from the flasks using a Trypsin-EDTA solution.

Cytotoxicity assay

Growth inhibition of tumour cells by obtained extracts was measured by a rapid colorimetric assay, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and compared with untreated controls (30). The assay is based on the metabolic reduction of soluble MTT by mitochondrial enzyme activity of viable tumor cells into an insoluble colored formazan product, which can be measured spectrophotometrically after dissolving in dimethyl sulfoxide (DMSO). Briefly, cells (1×10⁴ cells per each well) were seeded into 96-well microculture plates and allowed to adhere for 24 h before treatment. Then, each tumour cell line was exposed to extracts at 20, 60, 100, 250, 500, 1000, 1500, 2000, 2500 µg/ml concentrations for 72 h and each concentration was tested in triplicate. The first column of each microplate was assumed as negative control (containing no extracts). To assay the cell survival, 25 µl of MTT solution (5 mg ml⁻¹ in phosphate buffer solution) was added to each well and the plates were incubated for 3 h at 37 °C. Then, 200 µl of DMSO was replaced by DMSO and pipetted to dissolve any formazan crystals. The optical density (OD) was read on a Elisa reader (Microplate reader MR 600, Dynatech, USA) at a wavelength of 570 nm. The growth inhibitory activity of extracts against a variety of cancer cell lines expressed as IC₅₀ values (the extract concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells) were determined from dose response curves.

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). 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).

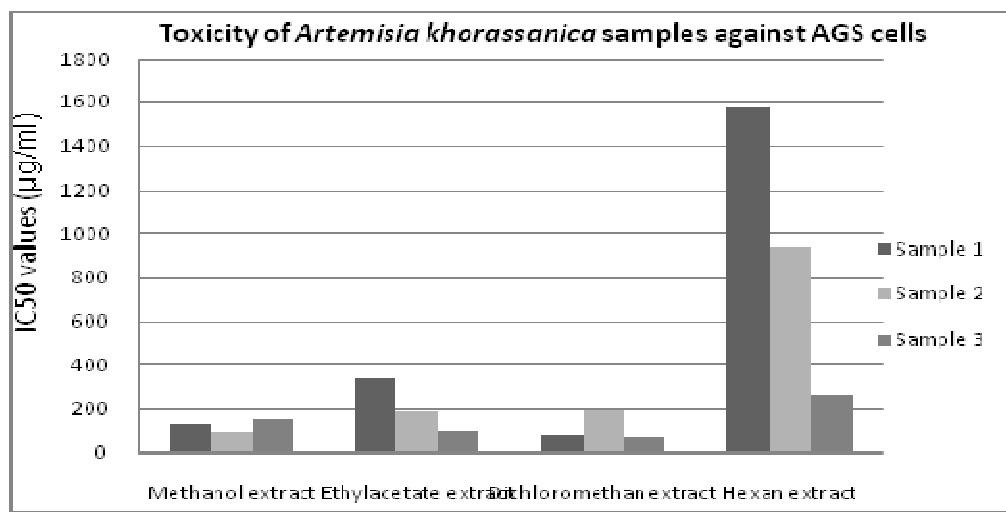
Results

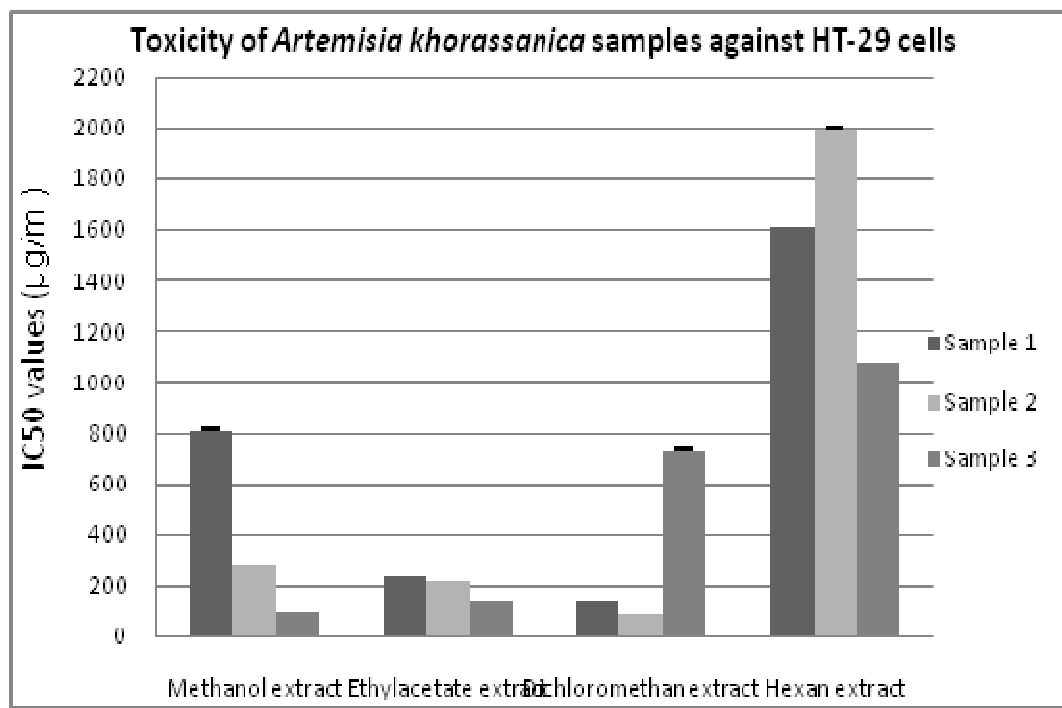
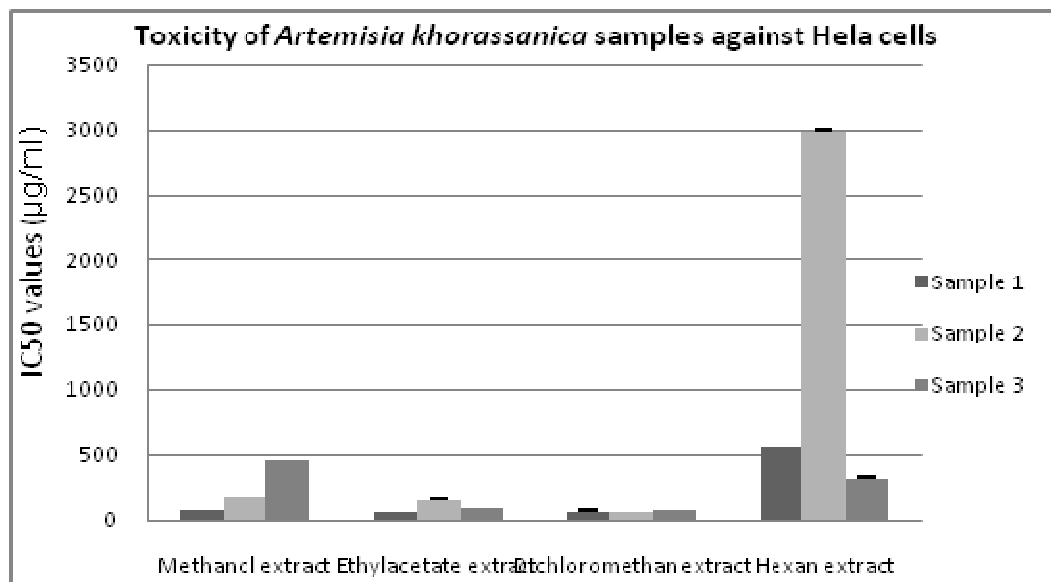
Results of the MTT cytotoxicity assay for methanol, ethylacetate, dichloromethane and hexan extracts of *Artemisia khorasanica* against AGS, Hela, HT-29 and MCF-7 cell lines are presented in figure 2. For sample 1, the calculated IC₅₀ values for methanol, ethylacetate, dichloromethane and hexan extracts against AGS cells were 131±1.2, 338±0.8, 79±0.3 and 1575±1.8 µg/ml; against Hela cells were 76±0.8, 63±0.8, 69±1.3 and 563±0.6 µg/ml; against HT-29 cells were 813±0.8, 237±0.8, 138±1.4 and 1613±2.3 µg/ml and 65±1.5, 56±0.8, 58±0.8 and 438±1.4 µg/ml against MCF-7 cells, respectively.

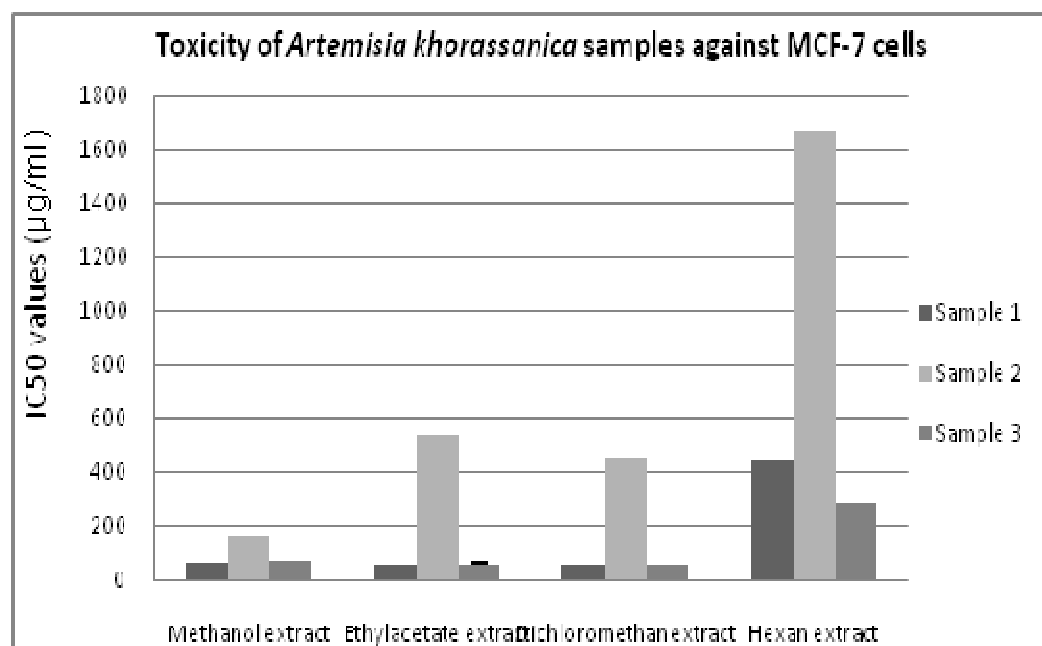
For sample 2, the calculated IC₅₀ values ± SEM for methanol, ethylacetate, dichloromethane and hexan extracts against AGS cells were 89±0.6, 190±2.1, 197±1.8 and 937±2.7 µg/ml; against Hela cells were 183±0.8, 153±2.7, 66±0.9 and 2993±3.5 µg/ml; against HT-29 cells were 276±1.8, 220±1.7, 89±1 and 2000±2 µg/ml and 161±1.5, 537±1.7, 451±1.6 and 1663±2.7 µg/ml against MCF-7 cells, respectively.

For sample 3, the calculated IC₅₀ values for methanol, ethylacetate, dichloromethane and hexan extracts against AGS cells were 152±1.2, 96±1.7, 67±1.5 and 263±2.3 µg/ml; against Hela cells were 465±1.2, 91±2, 77±1 and 312±1.7 µg/ml; against HT-29 cells were 97±1.3, 138±1.3, 738±1.1 and 1075±2.6 µg/ml and 76±1.6, 62±0.8, 57±1.2 and 288±1.2 µg/ml against MCF-7 cells, respectively.

Figure 2. Growth inhibition activity of different extracts from *Artemisia khorassanica* samples on AGS, Hela, HT-29 and MCF-7 cell lines were shown as IC₅₀ values (µg/ml) ± SEM.







Discussion

Methanol, ethylacetate, dichloromethane and hexane extracts from three samples of *Artemisia khorassanica* were screened against four human cancer cell lines: AGS (human gastric carcinoma cell line), Hela (human cervical carcinoma cell line), HT-29 (human colon carcinoma cell line) and MCF-7 (human breast carcinoma cell line). Growth inhibitory activity of different extracts was shown as IC_{50} from dose-response curves. As shown in figure 2, different extracts from *Artemisia khorassanica* samples inhibited growth of tested cancer cells.

For AGS, dichloromethane extract from sample 3 (IC_{50} : 67 $\mu\text{g/ml}$) and dichloromethane extract from sample 1 (79 $\mu\text{g/ml}$) showed the most inhibitory effects. The most inhibitory effects on Hela was for ethylacetate extract from sample 1 (IC_{50} : 63 $\mu\text{g/ml}$) and dichloromethane extract from sample 2 (IC_{50} : 66 $\mu\text{g/ml}$). For HT-29, dichloromethane extract from sample 2 (IC_{50} : 89 $\mu\text{g/ml}$) and methanol extract from sample 3 (IC_{50} : 93 $\mu\text{g/ml}$) had the most inhibitory effects. Ethylacetate and dichloromethane extract from sample 1 (IC_{50} : 56 $\mu\text{g/ml}$) and (IC_{50} : 58 $\mu\text{g/ml}$) and dichloromethane extract from sample 3 (IC_{50} : 57 $\mu\text{g/ml}$) showed its most strong effect on MCF-7 cell line (Figure 2).

Among different extracts from *Artemisia khorasanica* samples, dichloromethan extract from sample 1 showed strong inhibitory effect on various cancer cell lines. All hexan extracts showed the least growth inhibitory effect.

According to fure 2, MCF-7 was the most sensitive cell line to different extracts because the IC₅₀ values of most extracts on it were less than other cell lines. The IC₅₀ of ethylacetate extract of sample 1 for MCF-7 was 56 µg/ml but were 563 µg/ml for Hela, 1575 µg/ml for AGS and 1613 µg/ml for nHT-29 cells. HT-29 and Hela cell lines, as shown in table 2, had less cytotoxicity for tested extracts.

Taking together, the present study is the first to show toxicity of methanol, ethylacetate, dichloromethan and hexan extracts from *Artemisia khorasanica* samples in malignant cell lines. Based on obtained results, three samples from this herb showed different cytotoxicity patterns on cancer cell lines. So, we concluded that cultivation of a plant in different places could have affect its constituents. *Artemisia khorasanica* could be also considered as a promising chemotherapeutic agent in cancer treatment. Experiments for isolation and elucidation of anti-tumoral compounds from tested extracts is udergoing. It could provide further knowledge to mechanisms involved in this toxicity.

Acknowledgements

The authors would like to thank the authorities in research council of Mashhad University of Medical Sciences (MUMS).

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