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RESEARCH ARTICLE

Antiproliferative effects of extracts from Iranian *Artemisia* species on cancer cell lines

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Abstract

Objective: Different species of *Artemisia* (Asteraceae) have shown to exhibit antitumor activity. The aim of this study was to identify the antiproliferative effect of some *Artemisia* species from Iran on cultured human cancer cells.

Materials and methods: Methanol, ethyl acetate, dichloromethane and *n*-hexane extracts from aerial parts of seven species of *Artemisia* were prepared and their antiproliferative effects on four cancer (AGS, HeLa, HT-29 and MCF-7) and normal cell line (L929) were determined. Different concentrations of extracts were added to cultured cells and incubated for 72 h. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was employed to assess the cell viability.

Results: Different extracts exert various growth inhibitory effects. In case of AGS cells, dichloromethane and methanol extracts of *A. ciniformis* Krasch. & Popov ex Poljak. (IC_{50} values: 35 and 60 µg/ml, respectively) showed the highest growth inhibitory effects. HeLa cells were more sensitive to both *A. diffusa* Krasch. ex Poljak. dichloromethane (IC_{50} value: 71 µg/ml) and *A. ciniformis* ethylacetate (IC_{50} value: 73 µg/ml) extracts. Dichloromethane extracts of *A. diffusa*, *A. santolina* Schrenk and *A. ciniformis* (IC_{50} values: 42, 91 and 94 µg/ml, respectively) exhibited more inhibition on HT-29 cells in comparison to other extracts. MCF-7 cells were best inhibited by *A. ciniformis* dichloromethane (IC_{50} value: 29 µg/ml) and *A. vulgaris* L. ethyl acetate (IC_{50} value: 57 µg/ml) extracts.

Discussion and conclusion: This study shows the antiproliferative effects of *Artemisia* extracts on malignant cell lines. *Artemisia* could be also considered as a promising chemotherapeutic agent in cancer treatment.

Keywords: MTT assay; AGS; HeLa; HT-29; MCF-7

Introduction

Prevention of cancer is one of the most important public health and medicinal issues in this century. In past two decades, many natural products have been studied for their chemopreventive effects. Many plants with different pharmacological properties are known to contain a variety of chemical compounds that may have the potential for the prevention or treatment of malignancies (Rao et al., 2002; Zhang et al., 2002). Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and antitumoral activities. It has been shown that many extracts from natural products have positive effects against cancer, compared with chemotherapy or recent hormonal treatments (Pezzuto, 1997). Many plants have also been examined to identify new and effective anticancer compounds (Kim et al., 1998; Pietta et al., 1998; Swamy & Tan, 2000). Biological methods have been extensively employed to evaluate antitumoral activity of natural products in the past three decades.

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The genus *Artemisia* L. is one of the largest and most widely distributed of the Astraceae (Compositae). This genus is a large taxon, numbering over 400 species distributed mainly in the temperate zone of Europe, Asia and North America. These species are perennial, biennial and annual herbs or small shrubs. Leaves are alternate, capitulate small, usually race mouse, paniculate or capitate, inflorescence, rarely solitary. Involucral bracts in few rows. Receptacle flat to hemispherical, without scales and sometimes hirsute. Florets all tubular, achiness obvoid, pappus absent or sometimes a small scarious ring (Tutin & Persson, 1976; Podlech, 1986; Polyako, 1995; Heywood & Humphries, 1997; Mucciaralli & Maffel, 2002). The genus in Iran has 30 species, two of which are endemic to the country (Podlech, 1986; Ghahreman & Attar, 1999; Emami & Aghazari, 2008). Some chemical components of the genus include monoterpenes, sesquiterpenes, sesquiterpene lactones, flavonoides, coumarins, sterols, polyacetylenes (Tan et al., 1998; Mucciaralli & Maffel, 2002). Different species of *Artemisia* have a wide range of biological effects including antimalarial (Tan et al., 1998), cytotoxic (Zheng, 1994), antibacterial, antifungal (Tan et al., 1998), anti-inflammatory (Emami et al., 2010) and antioxidant (Cakir et al., 2005) characteristics.

This study was designed to determine the antiproliferative effects of methanol, ethylacetate, dichloromethane and *n*-hexane extracts from some *Artemisia* species of Iran on human cancer cells lines.

Materials and methods

Plant material

Seven species of *Artemisia* were collected from different regions of Iran (Figures 1 and 2). Dr. Mozaffarian, Research Institute of Forest and Rangelands, Ministry of Jahad- Keshavarzi, Iran, identified these species. Voucher specimens have been deposited in the Herbarium of School of Pharmacy, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. Related information on these plants and their names in Farsi is summarized in Table 1 (Mozaffarian, 2003).

Preparation of the plant extracts

Plant aerial parts of each species were shade-dried at room temperature (25–30°C) and later ground into a fine powder using a household blender and sieved with a 2 mm-diameter mesh. Pulverized materials (100 g) were extracted using a percolator with methanol and were then concentrated at 50°C under reduced pressure to dry. This material was then extracted three times with an equal volume of *n*-hexane to give an extract containing nonpolar compounds. Then, the solution was successively partitioned between dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc; Otsuka, 2006). These isolated extracts were dried. A partitioning scheme of the methanol extract of each *Artemisia* species is presented in Figure 3.

Cell cultures

Four different cell lines, AGS (human gastric adenocarcinoma cell line, NCBI#: C131), HeLa (human cervix carcinoma cell line, NCBI#: C115), HT-29 (human colon adenocarcinoma cell line, NCBI#: C466) and MCF-7 (human breast carcinoma cell line, NCBI#: C135) and L929 (mouse fibroblast-like cell line NCBI#: C161),

Figure 1. Images of tested *Artemisia* species.

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were obtained from National Cell Bank of Iran (Tehran, Iran). Cell lines were grown as a monolayer culture in Dulbecco modified Eagle's medium (DMEM) from Gibco Laboratories (Detroit, USA) and supplemented with 10% fetal bovine serum (Gibco Laboratories, Detroit, USA), 1% penicillin/streptomycin (100 IU/ml and 100 µg/ml, respectively) from Gibco Laboratories (Detroit, USA) in a 5% CO_2-humidified atmosphere at 37°C. For the experiments, cells were removed from the flasks using a 0.25% trypsin-EDTA solution.

In vitro proliferation assay

Growth inhibition of normal and tumor cells by obtained extracts was measured using a rapid 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma Chemical Co., St. Louis, MO) colorimetric assay (Mosmann, 1983) and compared with untreated controls. Briefly, cells (1×10^4 cells per each well) were seeded into 96-well microculture plates and allowed to adhere for 24 h before treatment. Then, each tumor cell line was exposed to extracts at 20, 40, 60, 80, 100, 250, 500, 750, 1000, 1500 and 2000 µg/ml concentrations for 72 h in triplicate. The first column of each microplate was assumed as negative control (containing no extracts). As obtained *Artemisia* extracts could interfere with MTT salts, the medium was replaced with fresh culture medium to add the MTT salts. Then, to assay the cell survival, 25 µl of MTT solution (5 mg/ml in phosphate buffered solution) was added to each well and the plates were subsequently incubated for 3 h at 37°C. Then, the produced formazan crystals were dissolved using dimethyl sulfoxide (DMSO). The optical density (OD) was read on a plate reader at 570 nm. The inhibitory rate of cell proliferation was calculated according to the following formula:

$$\text{Growth inhibition (\%)} = \frac{(OD_{control} - OD_{treated})}{OD_{control}} \times 100$$

The growth inhibitory activity of extracts against a variety of cancer cell lines expressed as IC_{50} values (the extract concentration reducing by 50% the absorbance in treated cells with respect to untreated cells) were determined from dose-response curves.

All treatments were done by setting up three independent experiments on separate days, each time with three or more wells for each concentration in 96-well plates.

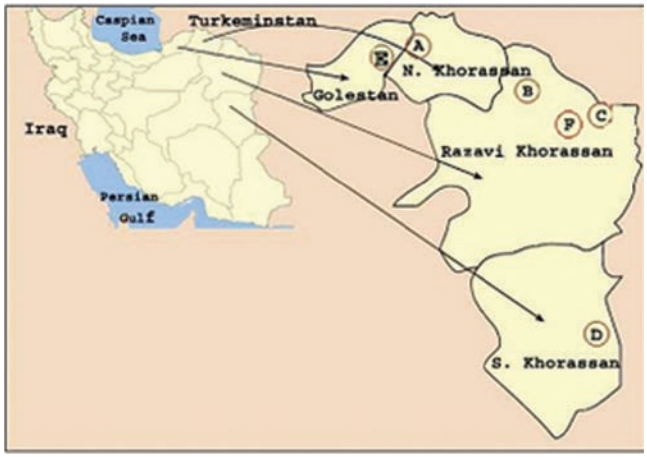

Figure 2. Geographical regions of collected *Artemisia* species from Iran. (A) *A. annua*, *A. ciniformis*; (B) *A. biennis*; (C) *A. diffusa*; (D) *A. santolina*; (E) *A. vulgaris*; (F) *A. persica.*

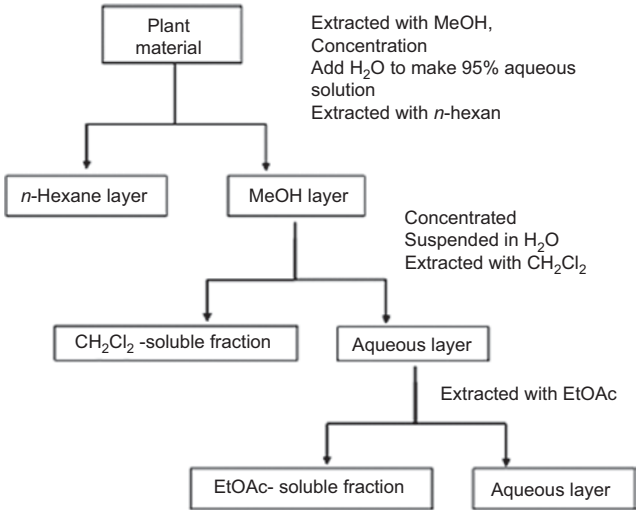

Figure 3. Partitioning scheme using immiscible solvent.

Table 1. Characteristics of tested *Artemisia* species.

Scientific name	Persian name	Location	Collection time
A. annua L.	Gandwash	Islamabad near Maraveh tapeh-Shahrabad road, North Khorasan province, height, 945 m	15 September 2007
A. biennis Willd.	Dermaneh Dosaleh	Near Chovailly-Bajgiran road, Ghochan, Razavi Khorasan province, height 1774 m	23 December 2004
A. ciniformis Krasch. & Popov ex Poljak.	Dermaneh Talaee	Maraveh tapeh-Shahrabad road, North Khorasan province, height 940 m	8 August 2003
A. diffusa Krasch. ex Poljak.	Dermaneh Afshan	Mazdavand, between Sarakhs and Mashhad, Razavi Khorasan province, height 940 m	19 August 2007
A. persica Boiss.	Dermaneh Denaee	Mshhad, Astan Gods Razavi farm	19 September 2007
A. santolina Schrenk	Dermaneh Sefid	Between Khosph-Birjand, Birjand, South Khorasan province, height 1460 m	20 September 2003
A. vulgaris L.	Berenjasf	Bojnord-Gorgan road, near Tangrah, Golestan province, Golestan forest, height 645 m	12 September 2007

Results

In this study, growth inhibitory activity of methanol, ethyl acetate, dichloromethane and *n*-hexane extracts from seven *Artemisia* species were screened against a panel of human cancer cell lines representing different histological types including AGS, HeLa, HT-29 and MCF-7 cell lines. Results are shown as IC_{50} values in Table 2.

For AGS cell line, isolated extracts inhibited the cell growth with IC_{50} values ranging from 35 μg/ml (for dichloromethane extract of *A. ciniformis* Krasch. & Popov ex Poljak.; Figure 4) up to more than 2000 μg/ml (for dichloromethane extract of *A. diffusa* Krasch. ex Poljak.). Methanol extract of *A. ciniformis* (IC_{50} value: 60 μg/ml) also had a high growth inhibitory effect on AGS cell line (Figure 4).

In case of HeLa cell line, obtained extracts showed growth inhibitory activity with IC_{50} values ranging from 71 μg/ml (for dichloromethane extract of *A. diffusa*; Figure 5) up to more than 2000 μg/ml (for methanol extracts of *A. diffusa*, *A. persica* Boiss, *A. santolina* Schrenk and *A. vulgaris* L.). It seems most methanol extracts had no remarkable inhibitory effect on the growth of HeLa cells. Ethyl acetate extract of *A. ciniformis*, dichloromethane extract of *A. biennis* Willd and dichloromethane extract of *A. ciniformis* (IC_{50} values: 73, 74 and 97 μg/ml, respectively) showed considerable growth inhibitory activity (Figure 5).

Dichloromethane extracts of *A. diffusa*, *A. santolina* and *A. ciniformis* (IC_{50} values: 42, 91 and 94 μg/ml, respectively) exhibited more inhibition on HT-29 cell line in comparison to other extracts (Figure 6). However, methanol extract of *A. santolina* (IC_{50} value: more than 2000 μg/ml) had the lowest inhibitory effect on HT-29 cells.

Although MCF-7 cells were best inhibited by *A. ciniformis* dichloromethane (IC_{50} value: 29 μg/ml) and *A. vulgaris* ethyl acetate (IC_{50} value: 57 μg/ml) extracts (Figure 7), it was not inhibited by *n*-hexane extract of *A. diffusa* (IC_{50} value: more than 2000 μg/ml).

Overall, among all tested extracts on cancer cell lines, *A. ciniformis* dichloromethane extract seems to be a strong inhibitor for the growth of a wide variety of tumor cell lines including AGS, HeLa, HT-29 and MCF-7 cell lines (IC_{50} values: 35, 97, 94 and 29 μg/ml, respectively).

We also examined normal fibroblast-like cell line (L929 cell line) for growth inhibitory activity of different extracts of *Artemisia* species. L929 cell growth was best inhibited by *A. ciniformis* methanol and dichloromethane extracts (IC_{50} values: 79 and 82 μg/ml, respectively; Figure 8). In addition, *A. diffusa n*-hexane extract (IC_{50} value: 84 μg/ml) showed high inhibition on the growth of L929 cells.

Table 2. Growth inhibition activity of different extracts from *Artemisia* species on AGS, HeLa, HT-29 and MCF-7 cancer cell lines. Data are shown as IC_{50} values (μg/ml).

Artemisia spp. extracts	AGS	HeLa	HT-29	MCF-7	L929
A.annua (Methanol)	105	360	365	130	566
A.annua (Ethyl acetate)	912	1675	1325	850	1952
A.annua (Dichloromethane)	76	388	510	228	605
A.annua (*n*-Hexane)	161	168	205	130	221
A.biennis (Methanol)	525	288	925	451	401
A.biennis (Ethyl acetate)	963	1050	1100	1224	1350
A.biennis (Dichloromethane)	337	74	337	451	902
A.biennis (*n*-Hexane)	463	301	131	338	538
A.ciniformis (Methanol)	60	130	388	73	79
A.ciniformis (Ethyl acetate)	750	73	1252	64	168
A.ciniformis (Dichloromethane)	35	97	94	29	82
A.ciniformis (*n*-Hexane)	205	300	337	363	498
A.diffusa (Methanol)	338	>2000	851	115	1200
A.diffusa (Ethyl acetate)	325	154	155	103	153
A.diffusa (Dichloromethane)	>2000	71	42	88	388
A.diffusa (*n*-Hexane)	138	205	375	>2000	84
A.persica (Methanol)	675	>2000	688	438	198
A.persica (Ethyl acetate)	160	248	253	376	275
A.persica (Dichloromethane)	800	219	205	812	257
A.persica (*n*-Hexane)	337	138	113	301	188
A.santolina (Methanol)	1226	>2000	>2000	526	1276
A. santolina (Ethyl acetate)	713	221	301	387	288
A. santolina (Dichloromethane)	153	538	91	364	313
A. santolina (*n*-Hexane)	145	357	130	76	539
A.vulgaris (Methanol)	588	>2000	1850	551	913
A.vulgaris (Ethyl acetate)	505	387	505	57	538
A.vulgaris (Dichloromethane)	563	351	363	1125	755
A.vulgaris (*n*-Hexane)	153	160	288	1951	451

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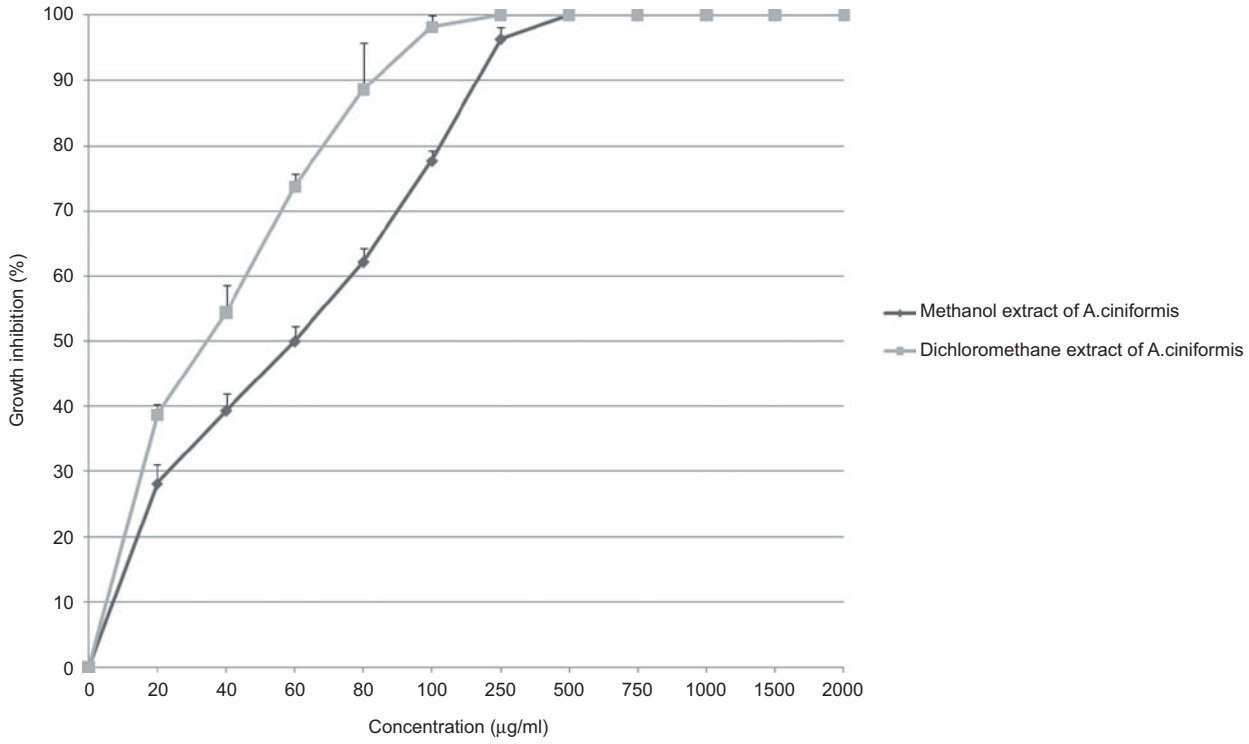

Figure 4. Antiproliferative effect of methanol and dichloromethane extracts from *A. ciniformis* on AGS cancer cell line.

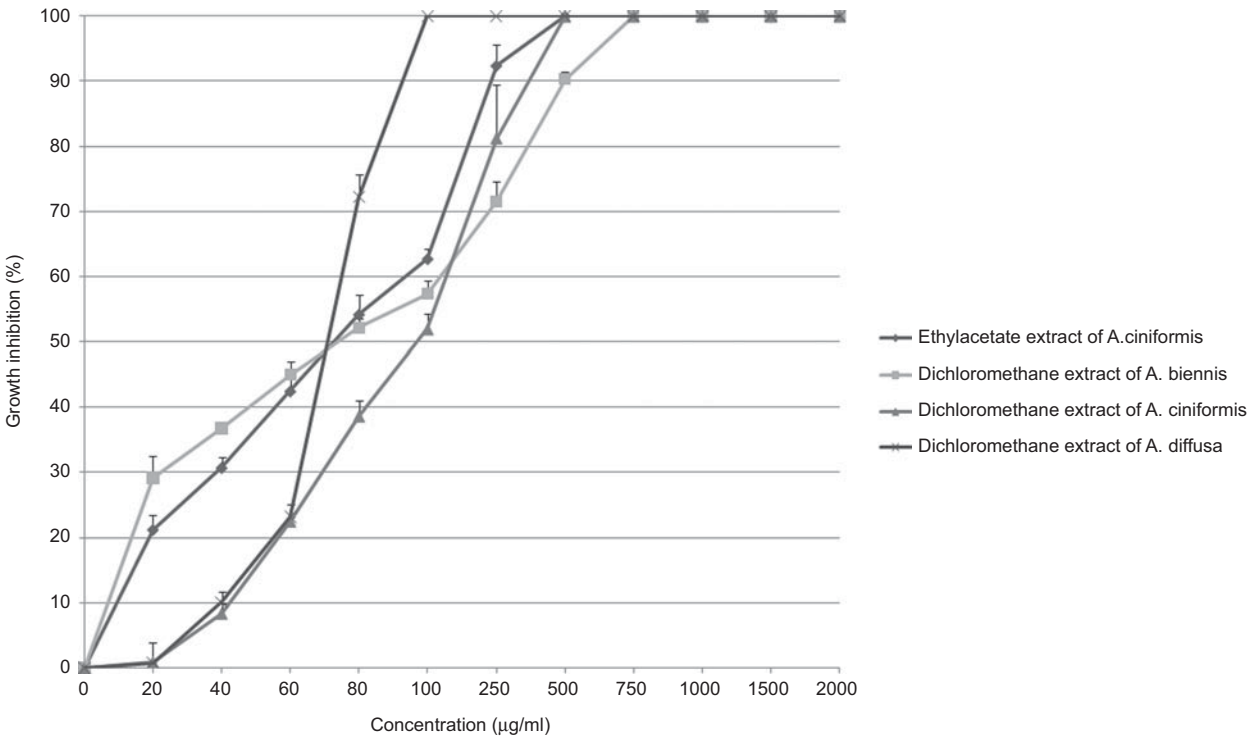

Figure 5. Antiproliferative effect of dichloromethane extracts from *A. diffusa*, *A. biennis*, *A. ciniformis* and ethyl acetate extract from *A. ciniformis* on HeLa cancer cell line.

Discussion

Cancer is a rising health problem around the world. Natural products have long been used to prevent and treat many diseases, including cancer, and thus, they are considered suitable candidates for the development of anticancer drugs (Smith-Warner et al., 2000).

Various reports have been published on antiproliferative activity of different *Artemisia* spp. fractions and/or extracts. Essential oil of *Artemisia annua* could induce

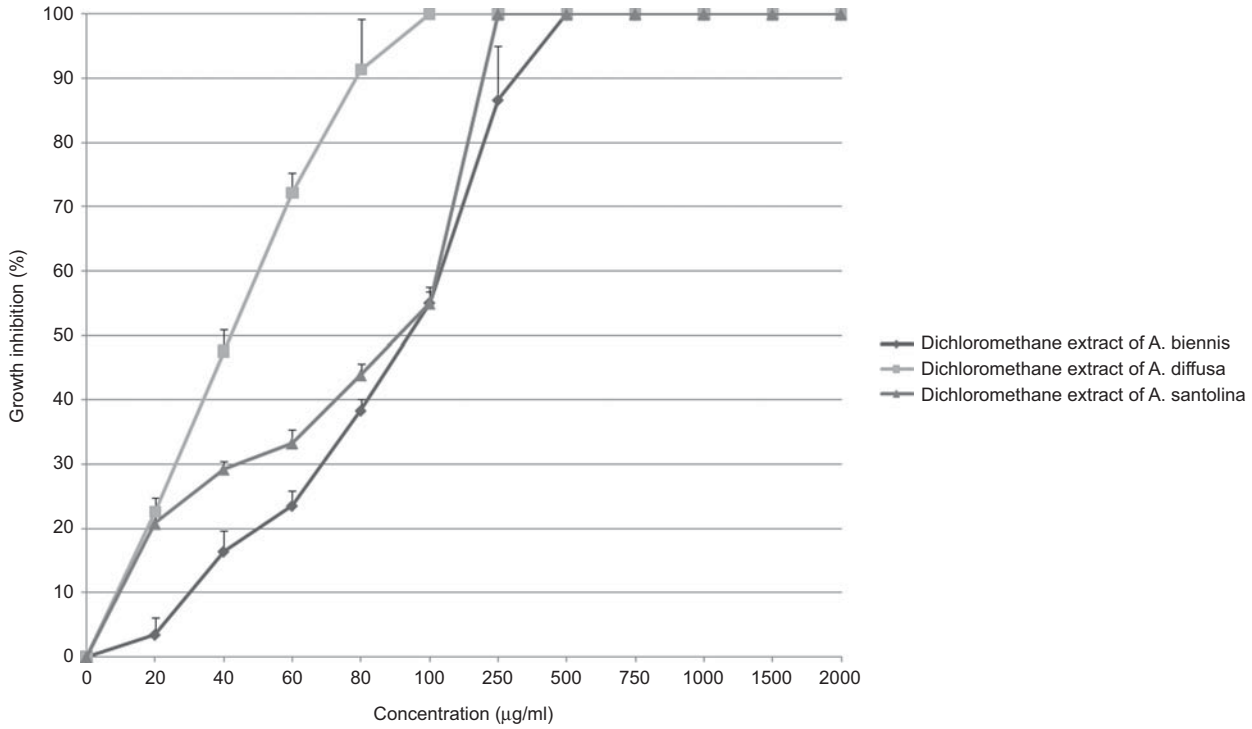

Figure 6. Antiproliferative effect of dichloromethane extracts from *A. diffusa*, *A. ciniformis* and *A. santolina* on HT-29 cancer cell line.

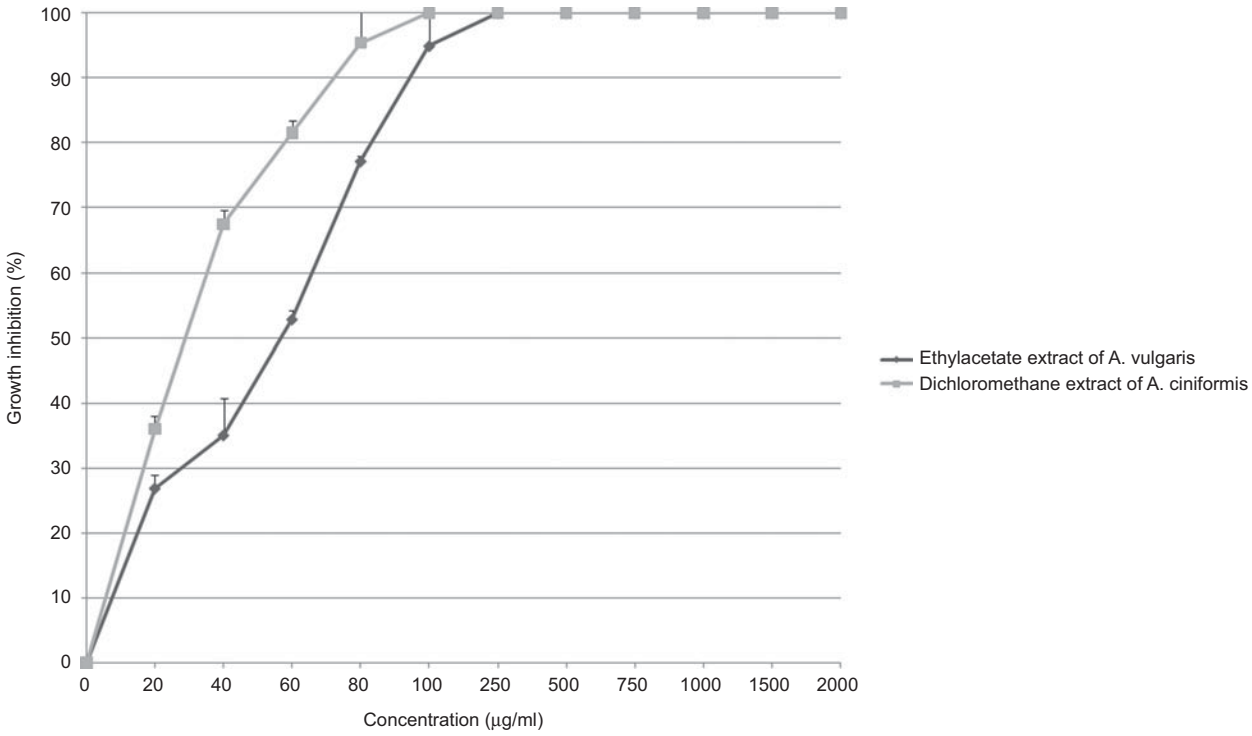

Figure 7. Antiproliferative effect of dichloromethane extract from *A. ciniformis* and ethyl acetate extract from *A. vulgaris* on MCF-7 cancer cell line.

apoptosis of cultured SMMC-7721 cells. Artemisinin, a compound isolated from the sweet wormwood (*A. annua*), has shown to have selective toxicity toward cancer cells *in vitro*. Moreover, it is given orally to retard breast cancer development in 7,12-dimethylbenz[α] anthracene (DMBA)-treated rats (Li et al., 2004; Henry & Narendra, 2006). Jaceosidin was isolated from *Artemisia argyi* as a putative oncogene inhibitor. It might be used as a potential drug for the treatment of cervical cancers associated with human papillomavirus and ovary cancer (Lee et al., 2005; Lv et al., 2008). Whole herbal extracts of *A. asiatica* has been used in oriental traditional medicine

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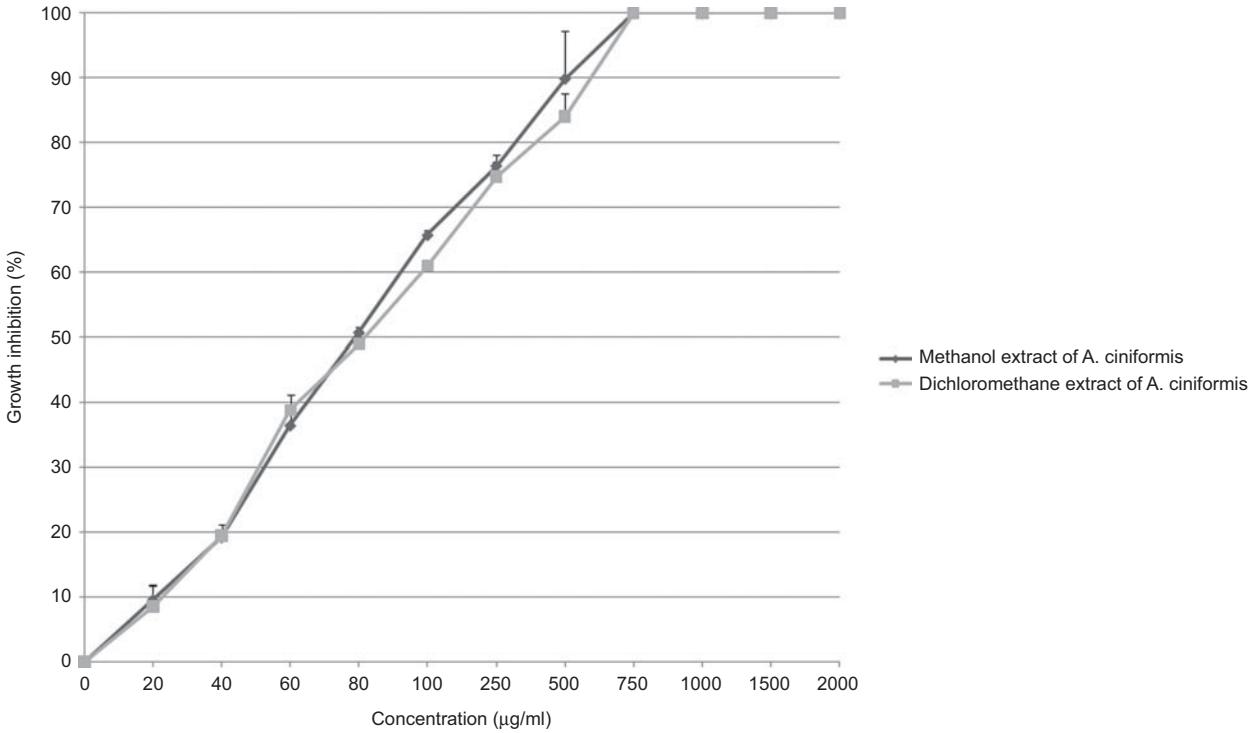

Figure 8. Antiproliferative effect of methanol and dichloromethane extracts from *A. ciniformis* on normal fibroblast-like L929 cell line.

for the treatment of inflammation, cancer and other disorders. Eupatilin, a pharmacologically active ingredient of *A. asiatica*, exhibited antiproliferative effect on HL-60 and AGS cells (Seo & Surh, 2001; Kim et al., 2005). The ability of *A. princeps* smoke and water extracts to induce apoptosis was evaluated *in vitro* on human breast cancer MCF-7 cells. Its smoke and water-soluble extracts induce apoptosis via the mitochondrial pathway in breast cancer cells (Sarath et al., 2007). Yomogin is an active compound isolated from *A. princeps*, a traditional oriental medicinal herb, which has been shown to inhibit the proliferation of human promyelocytic leukemia cells through the induction of apoptosis (Jeong et al., 2004). Aqueous and methanol herbal extracts from *A. capillaries* and *A. annua* L. showed inhibitory activity against different cancer cell lines (Sun et al., 2007). Cirsilineol, a compound isolated from *A. vestita*, inhibits proliferation of cancer cells by inducing apoptosis via mitochondrial pathway (Sheng et al., 2008). Smoke and water-soluble extracts from dried leaves of *A. princeps* induce apoptosis via mitochondrial pathway in human breast cancer MCF-7 cells (Sarath et al., 2007). DA-9601, a standardized extract of *A. asiatica*, blocked TNF-α-mediated inflammatory signals by potentially modulating the p38 kinase pathway and/or a signal leading to NF-κB dependent pathways in gastric epithelial cells (Choi et al., 2006).

Here, we report antiproliferative effects of some *Artemisia* species from Iran against different cancer cell lines. Although these species have been used in folk medicine, however, this is the first report of their antiproliferative activity. All *Artemisia* extracts showed degrees of inhibitory effects on cultured cancer cell lines. For AGS, dichloromethane and methanol extracts from *A. ciniformis* showed the highest inhibitory effects. The highest inhibitory effects on HeLa were for dichloromethane extract from *A. diffusa* and ethyl acetate extract from *A. ciniformis*. For HT-29, methanol extract from *A. annua* and dichloromethane extract from *A. diffusa* had the highest inhibitory effects. Dichloromethane extract from *A. ciniformis* and ethyl acetate extract from *A. vulgaris* showed the highest inhibitory effect on MCF-7 cell line.

Among different extracts from studied *Artemisia* species, dichloromethane extract from *A. ciniformis* showed the highest overall inhibitory effect on various cancer cell lines.

Conclusion

This study is the first to show the antiproliferative activity of different extracts from various *Artemisia* species collected in Iran. These extracts showed varied inhibitory effects on different cancer cell lines. Based on the overall strong inhibition effects of *A. ciniformis* dichloromethane extract, isolation and studying of its compounds is suggested.

Declaration of interest

This study was supported by a grant (No. 86468) from Mashhad University of Medical Sciences (MUMS) vice president for research, Mashhad, Iran.

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