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The Antiproliferative Effects of Petroleum Ether Extract of *Artemisia Aucheri* on Human Cancerous Cell Lines

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Different species of *Artemisia* (Asteraceae) have shown to exhibit antitumor activity. The aim of this study was to identify the anti-proliferative effect of *Artemisia aucheri Boiss (A.aucheri)* from Iran on cultured human cancer cells. Aerial parts of *A. aucheri* Boiss. were collected from Chahar Bagh region , Golestan province, Iran. The dried powdered aerial parts of *A.aucheri* were extracted with petroleum ether (40-60), dichloromethane, ethyl acetate, ethanol and ethanol-water (1:1 v/v), respectively. For biological investigation different concentrations of extracts were added to cultured cells and incubated for 24 h. MTT assay was employed to assess the cell viability. In addition, morphological changes of cells were assayed using phase contrast inverted microscope. Different extracts exert various growth inhibitory effects. In case of SKNMC, MCF-7 and A2780 cell lines, petroleum ether extract (IC_{50} values: 21.0, 26.0 and 28.0 µg/ml, respectively) showed the highest growth inhibitory effects. Dichloromethane extract showed IC_{50} values: 27.0, 35.0 and 55.0 µg/ml, respectively.This study showed the anti-proliferative effects of *A.aucheri* petroleum ether extract on malignant cell lines. Thus, the aforementioned may be considered as a promising chemotherapeutic agent in cancer treatment.

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Introduction

Plants have been a major source of very effective drugs which are used for treatment of various forms of cancer. Moreover, the actual compounds isolated from the plant provide leads for the development of potential novel agents [1].*Artemisia* species (*Asteraceae* family) comprises 1310 genera with about 13,000 species worldwide. *Asteraceae* are commonly used in folk medicine, approximately 300 species have been reported to have ethno-medical uses for the treatment of cancer .Numerous experimental studies have demonstrated that some *Asteraceae* species exert their anti-tumour activity due to the presence of flavonoids, sesquiterpenelactones,lignans, acetylenes, triterpenes or glycolipids[2].*Artemisia aucheriBoiss (A.aucheri)*, is an indigenous plant that can be found across Iran. In traditional medicine, A.aucheri is used for its astringent, disinfectant, antimicrobial and antiparasiticproperties [3]. Fifty-four components were identified in the essential oil of *A.aucheri*, that major components were geranyl acetate (17.2%), E-citral (17.1%), linalool (12.7%), geraniol (10.7%) and Z-citral (10.5%). 1,8-cineole (2.1%), borneole (2.5%), camphor (3.2%) are other major components[4]. Despite the fact that many publications have studied the compositions of *A.aucheri*, no report on its cytotoxic effect is yet available. The present study was aimed to evaluate and compare the cytotoxic effects of different extractions of *A.aucheri* in three human carcinoma cell lines including: SKNMC (human neuroblastoma), MCF-7 (human breast cancer cells) and A2780 (human ovarian cancer cells).

Materials and Methods

Reagents and Chemicals

Dulbecco's Modified Eagle's Medium (DMEM) and Fetal Bovine Serum (FBS, heat inactivated), penicillin, streptomycin were purchased from Gibco (Grand Island,USA). Trypsin-EDTA, phosphate buffer saline (PBS), dimethyl-solfoxide(DMSO), 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT)were purchased from Sigma (StLouis, MO, USA). All solvents used for extraction were purchased from Caledon and Scharlau.

Plant material

Aerial parts of *A.aucheri* were collected from ChaharBagh region (2150 m altitude, Sep. 2011), Golestan province, Iran. Sample was identified by Mr. S. A. Hosseini (Agricultural and Natural Resources Research Center of Golestan Province, Gorgan, Iran) where voucher specimen (No. 2383) had been deposited in the herbarium.

Preparation of Extracts

The dried powdered aerial parts (100 g) of *A.aucheri* were extracted with petroleum ether (40-60), dichloromethane, ethyl acetate, ethanol and ethanol-water (1:1 v/v) respectively (Sequential maceration with ca. 3×1 L of each solvent).The extracts were filtrated and dried using reduced pressure rotary evaporator at a temperature below 45 $^{\circ}$**C** to yield 1.17, 4.52, 0.50, 9.16 and 7.33 g of each extract, respectively.

Cell culture conditions

SKNMC, MCF-7 and A2780 cell lines were obtained from Pasteur Institute (Tehran, Iran) and maintained at 37°C in a humidified atmosphere (90%)containing 5% CO_2. Cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM-F12) with 10% (v/v) fetal bovine serum, 100Uml^{-1} penicillin and 100 µgml^{-1} streptomycin. The cells were cultured in 25 cm^3 tissue culture flasks (Nunc, Denmark), and incubated in a Memmert water-jacketed CO_2 incubator. Cells were observed routinely under inverted microscope (Motic AE31, China) for proliferation and any contamination.

Assessment of cell proliferation

SKNMC, MCF-7 and A2780 cells (15×10^3cells/well) were seeded in triplicate into 96 well tissue culture plates (Becton Dickinson, USA) and incubated overnight. The *A.aucheri* extracts were diluted to desired concentrations using DMSO (64.16, 128.31, 192.47, 256.62 and 320.78µg/ml).For each concentration and time course study, there was a control sample that remained untreated and received the equal volume of solvent.

After 24 h of incubation, the medium was removed and 0.1 mg/well of MTT was added to cells, and plates were further incubated for 3 h at 37°**C**.The formazan crystals formed were solubilized using 100 µL of DMSO. Absorbance was determined on an ELISA plate reader (BioTek, USA) with a test wavelength of 570 nm and a reference wavelength of 630nm to obtain sample signal (OD_{570}-OD_{630}). Percentage of proliferation was calculated using the following formula: Percent of control proliferation= (OD test/$OD_{control}$) ×100. IC_{50} values were calculated by plotting the log10 of the percentage of proliferation versus drug concentration.

Morphological changes using phase contrast inverted microscope

Observation of morphological changes of cells was performed according to a previous study[12]. Briefly, 5 $\times 10^5$ cells were incubated for 24 h with or without selected compounds at IC_{50} concentration in a six well tissue culture dishes. The medium was discarded and cells were washed once with PBS. The morphological changes of the cells were observed using phase contrast inverted microscope (Motic, China) at 20x magnifications.

Statistical analysis

Each experiment was performed at least three times, and the results were presented as mean± SD. One-way analysis of variance (ANOVA) followed by Turkey's test was used to compare the differences between means. A probability value of $p< 0.05$ was considered to be statistically significant.

Results

Cytotoxicity Screenings of the crude extracts of A.aucheri

The cells were treated with different concentrations of *A.aucheri* (10-367.85 µg /ml). After 24 h the rate of cell growth inhibition was evaluated by MTT assay. Complete dose-response curves were generated and IC_{50} values were calculated, averaged from three experiments, against three human cancer cell lines. As shown in (Fig1) the crude petroleum ether extract showed good inhibitory effect , and exhibited potent cytotoxic activity with IC_{50} values 21.0, 26.0 and 28.0 µg/ml against SKNMC A2780 and MCF-7 respectively . The dichlromethan extract of *A.aucheri* possessed good inhibitory effect against SKNMC (IC_{50} values 27.0 µg/ml) and moderate effect against MCF-7 and A2780 (IC_{50} values 35.0 and 55.0 µg/ml, respectively), whilst the ethanolic extract showed weak cytotoxicity against all tested cell lines with IC_{50} values of >100.0, 77.0 and 16.0 µg/mL.The ethyl acetate and ethanol-water (1:1 v/v) extracts were not cytotoxic against any of the tested cell lines. (Table 1)

Table 1. Cytotoxic activity [(IC_{50} values (µg/ml)] of crude extracts of *A.aucheri* against human carcinoma cell lines.

Extracts	yield (g)	IC50 (µg/ml) SKNMC	MCF-7	A2780
PE	4.01	21±1.06a	26±0.98	28±1.74
DCM	27.0	27±2.49	35±3.81	55±4.05
EA	0.50	>100	>100	>100
EtOH	9.16	>100	77±6.52	16±1.59
EtOH/Wt	7.33	>100	>100	>100

a) Data are expressed as the Mean±SD of three separate experiments (n= 3).

Morphological Changes during petroleum ether extract treatment

Morphological changes in treated cells with extracts for 24h were observed (Fig 2) in comparison to control cells. Visualization of the control (untreated) cells indicated that the cells maintained their original morphology form containing several nucleoli. Most of the control cells were adherent to the tissue culture flasks. After 24 h treatment with IC_{50} concentration of petroleum ether extract, cell population was significantly decreased compared to control. Moreover in petroleum ether extract treated cells, moderate cytoplasmic granulations were observed. Also cells became rounded and started to detach from the wells (Fig 2A–C).One day after treatment, nearly 90% of surface in the control flasks were confluent, while in petroleum ether extract treated cells only 50-60 % of the surfaces were covered by cells.

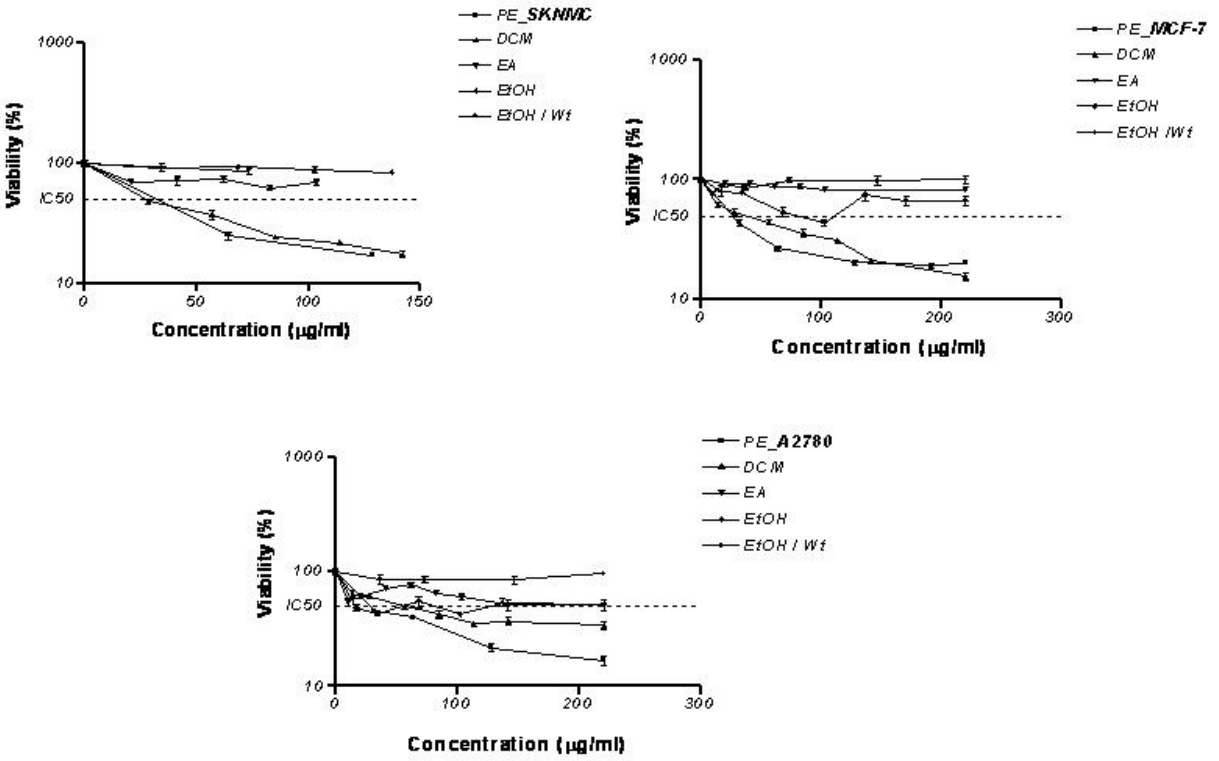

Fig. 1. Dose-dependent growth inhibition of malignant cell lines by total petroleum ether extract(10-367.85 µg/ml) after 24 h. Viability was quantitied by MTT assay. Dose inducing IC_{50} was calculated. [PE: Petroleum ether, DCM : Dichloromethane, EA : Ethyl acetate, EtOH : Ethanol ,EtOH/Wt : Ethanol-water (1:1 v/v)].Data are expressed as the Mean±SD of three separate experiments (N= 3).

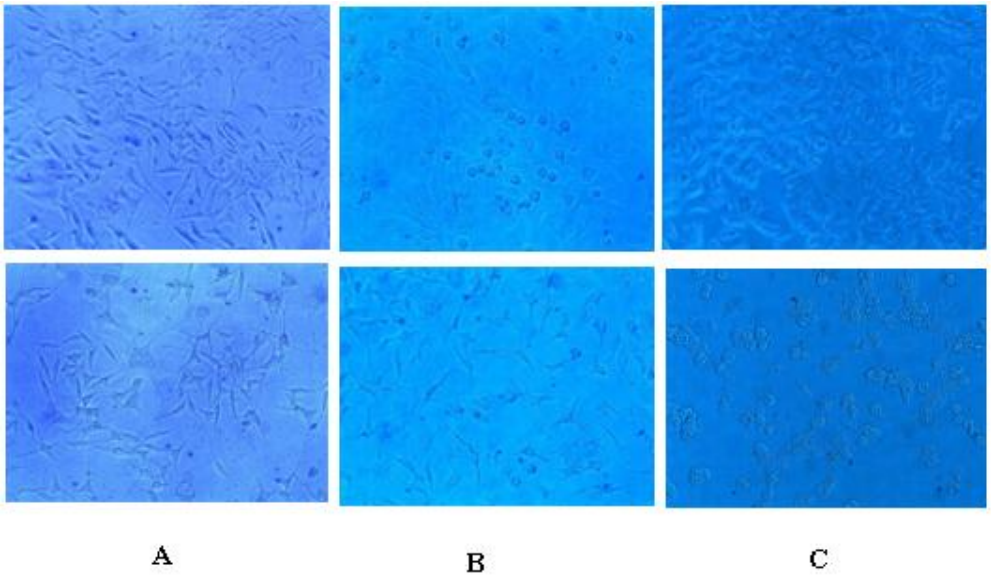

Fig. 2. Representative photomicrograph shows morphological changes of human cancer cells A)SKNMC, B)A2780 and C)MCF-7.Cells were treated with PE extract for 24 h and imaged by inverted phase contrast microscope. Light microscopic images of cells show morphological changes after treatment (20X magnification).

Discussion

Previous studies have shown the anti-proliferative activity of *Artemisia* species including *A. princeps*, *A. absinthium*, *A.verlotorum* and *A.annua*[5-8].However, no data is available regarding the cytotoxic effect of *A.aucheri* on cancerous cell lines. Therefore, in this study we investigated the cytotoxic effects of aerial parts of *A.aucheri*. Using MTT assay, the effects of the various extracts on the viability of different cell lines was examined. In current study the most potent effect was exerted by petroleum ether following dichlromethan extracts. Moreover, SKNMC cells were found to be the most sensitive to petroleum ether (50% viability at 21 µg/ml) and dichlromethan (50% viablity at 27 µg/ml) followed by MCF-7 and A2780 cell lines. Therefore, mixture of phytochemicals extracted by non-polar solvents (petroleum ether and dichlromethan) showed significant cytotixicity on the cell lines tested compared with those were present in more polar extracts (Ethyl acetate, Ethanolic and ethanol-water). According to the NCI (United States National Cancer Institute of Plant Screening Program), petroleum ether extract considered to have active cytotoxic effect because it has an IC_{50} value around 20 µg/ml[9-12]. The petroleum ether extract is very poor in phenolic compounds and mainly enriched in non-polar constituent such as tocopherols and tocotrienols, sterols and fatty acids [13].

Previous studies showed that dimeric guaianolides and eudesmane sesquiterpenoids from *A.anomala* exhibited cytotoxicity against different cell lines [14,15].α-methylene-γ-lactones isolated from *A. myriantha* and *A. absinthium* have been reported to possess anticancer potential[16]. Flavonoids [17] and sesquiterpene courmarin ethers are other examples of cytotoxic compounds which isolated from the genus of Artemisia [18].

The positive result of crude petroleum ether extract of aerial part of *A.aucheri* suggests that the plant may contain antitumor compound. Thus, it may be considered as a promising chemotherapeutic agent in cancer treatment.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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