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“Study on cytotoxic activity of chloroformic fractions from Astraceae family on a number of cancer cell lines”

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Study on cytotoxic activity of chloroformic fractions from Astraceae family on a number of cancer cell lines

Abstract
Cancer is considered as one of the leading causes of death worldwide. The standard treatments of cancer are surgery, chemotherapy and radiation therapy. It is significant that a number of currently used anti-cancer agents are derived from natural sources, including plants, marine organisms and micro-organisms. In Iran, because of its climate diversity numerous varieties of plants can grow. Many of these plants such as Glycyrrhizaglabra, Foeniculumvulgare and Polygonumspecies have shown to possess anti-inflammatory and immunomodulatory effects. Asteraceae or Compositae family is famous for its tranquilizing effect, antifungal and cytotoxic activities. In frame of an ethnopharmacological project, local healers of some provinces of north east of Iran were interviewed by using questionnaire forms and could identify a number of herbs mainly from Astraceae family which are used for treatment traditionally in some areas of northern part of Iran. To screen the anti cancer effects of plants from this family, plants were collected and extraction was done by using methanol maceration and finally extracts were tested for their toxicity toward a number of cancer cell lines by performing colorimetric cytotoxicity assay, extracts with high toxicity were sent for fractionation. The current study was aimed to identify the possible cytotoxic effect of two chloroformic fractions from Asteraceae family on four cancer cell lines (HepG2, HeLa, MN1 and MDD2) by using MTT colorimetric cytotoxicity assay. Results suggest that following 72 hours exposure, both fractions exhibited a substantial antiproliferative effect in all four tested cell lines. Moreover, concentration range for inducing 50% of cell death (IC$_{50}$%) was determined. Our results point to a robust inhibitory effect of chloroformic fractions specifically toward HeLa cancer cell lines. These plants represent valuable resources for the development of potential anticancer agents.

Keywords
Cytotoxicity, Asteraceae, Anticancer.
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Introduction

Cancer is a term for a large group of diseases that can affect any part of the body. Other terms used are malignant tumors and neoplasm. One crucial feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. This process is referred to as metastasis. The transformation from a normal cell into a tumor cell is a multistage process, typically a progression from a pre-cancerous lesion to malignant tumors (WHO Fact sheet, 2011). The standard treatments of cancer are surgery, chemotherapy, radiation therapy, targeted therapy, immunotherapy, anti-angiogenesis therapy, hyperthermia, photodynamic and laser therapy (American Cancer Society, 2011).

It’s significant that over 60% of currently used anti-cancer agents are derived in one way or another from natural sources. Indeed, molecules derived from natural sources, including plants, marine organisms and micro-organisms played an important role in the discovery of leads for the development of conventional drugs for the treatment of most human diseases. Plant-derived compounds have been an important source of several clinically useful anti-cancer agents; these include vinblastine, vincristine which are camptothecin (cytotoxic quinoline alkaloid) derivatives and used to treat certain kinds of cancer (Cragg and Newman, 2004). Plants belonging to the family Asteraceae found to be one of the largest plant families. It contains over 40 economically important species; they are used as food, (lettuce and jerusalem artichoke) oil (sun flowers and safflower), medicine (chamomile) and many as ornamental plants (Burkill, 1985) (Kasim et al., 2011). Studies show that the oils from Asteraceae plant family are active against A. fumigates (Zapata et al., 2010); moreover, fractions of different leaf extracts of B. pilosa from Asteraceae family showed potential in vitro anticancer and antimalarial activity (Kumari et al., 2009). Studies show that leaves of Struchiumsparganophora from Asteraceae plant family have nutritive, antioxidant, antimicrobial and anti-malaria activities (Kasim et al., 2011).

In Iran numerous varieties of plants grow and at least 1000 species are recorded as medicinal plants. Many of these plants such as Punicagranatum, Glycyrrhizaglabra, Foeniculumvulgare and Polygonumspecies prescribed by ancient Iranian local healers have been shown to possess anti-inflammatory and immunomodulatory effects (Amirghofran, 2010).
In frame of an ethnopharmacological project, local healers of some provinces were interviewed by using questionnaire forms and botanists could successfully identify a number of herbs from Asteraceae family which are used for anti-inflammatory and antibacterial treatment traditionally in the area. While these herbs have been used traditionally in the provinces as anti-inflammatory agent, studies directed specifically towards possible mechanism of action of these herbs have not been performed.

In an effort to identify potential cytotoxic activity of this family of plants, we conducted the present study to establish an evidence of cytotoxic activity of chloroformic fractions from Asteraceae family on a number of cancer cell lines.
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**Methods**

Plant materials:
Plants were collected in 2007 in the town called Turkmen Sahra, located at northeast of Iran near the Caspian Sea. The plant materials were identified by Dr. Pirani and confirmed by Dr. Naghibi at ITMRC Herbarium in Traditional Medicine and Materia Medica research Center (ITMRC) affiliated to Shahid Beheshti University of Medical Sciences. Voucher specimens (Ap 207 FoL) and (Ap 208 Fs) have been deposited in the herbarium of the ITMRC Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Preparation of methanolic extracts:

The plant material were dried and then ground and stored. The method for preparing extracts involved stirring the ground plant (10g) in methanol (50ml) over night. The extracts were then concentrated and stored in 4°C until use (Sahranavard et al., 2009).

Fractionation:

Fractionation was done by maceration of methanol extracts of plants respectively with (petroleum ether, hexane and chloroform) each (0.1) gr/ml for 24h. Fractions were immediately concentrated and stored in 4°C until use. The biological tests were carried out using the crude dry fractions.

Cell culture:

Cancer cells were obtained from National cell bank of Iran (NCBI), Pasteur Institute, Tehran, Iran and maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) (Sigma-Aldrich, St. Louis, MO) supplemented with 10% foetal bovine serum and maintained at 37°C in a 5% CO₂ incubator. After incubation, growth medium was removed and cells were rinsed with PBS (phosphate buffer saline), 1X. Cells were then harvested by trypsinization and then centrifuged at 500 x g for 5 min at 4°C. The pellet was washed with PBS 1X and resuspended with 1 ml of culture medium. An aliquot was used to assess the cell counting by using...
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hemocytometer cell count. Cells were plated (5*10^4 cell/well) in 96-well plate and incubated for 24 hours at 37°C until they reached to about 80% confluence.

Cell proliferation assay:
The cell proliferation was assessed by the metabolism of 3- (4, 5-dimethylthiazol-2yl)-2, 5-biphenyl tetrazolium bromide to form an insoluble formazan precipitated by mitochondrial dehydrogenases that only present in viable cells (Thabrew et al., 2005). In short, after cells were plated onto 96-well tissue culture plates and incubated overnight to reach the density of 5*10^4 cells/well in 100 mL of aforementioned media. After 24 hours of culture, the medium was removed carefully and replaced with medium with various concentrations of fractions from highest 100 µg/ml to the lowest 0.781 µg/ml and incubated for additional 72 hours. DMSO (Dimethyl sulfoxide) was used as negative control and Cadmium chloride 30 % was applied as positive control. Subsequently, 20 µl of MTT solution (5 mg/mL) was added in each well of the 96-well plate, and plate incubated at 37°C for 4 hours then medium was removed by aspiration and 150µl DMSO was added per well. The plate was shaken for 15 min and the absorbance at 590 nm measured using ELISA micro titer plate reader. For each experiment, three identical wells were used for each concentration.

Statistical analysis:
Statistical analysis was performed using the Graph pad Prism computer software (version 06). The results were expressed as the mean SD of three repeat of each concentration made in one experiment. All data were analyzed using (One-Way ANOVA). All post hoc comparisons were made using a Dunnett's test when significant ANOVA effects were determined. The threshold for statistical significance was set as P ≤ 0.05.
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**Results**

In this study, chloroformic fractions of two plants were prepared and used for in vitro experiments. Four different cancer cell lines were exposed to varying concentrations of each plant fraction for 72 hours and the cytotoxicity was measured by the MTT assay. The results of MTT test of fractions on cancer cell lines is indicated in Table 1. Chloroformic fractions from Asteraceae family reduced the viability and proliferation of cancer cells (HeLa, HepG2, MN1 and MDD2) in a concentration-dependent manner (Table 1).

Fraction number (207) has shown inhibitory effect toward (HeLa) cells between (100-50) (µg/ml). Aforementioned fraction was highly toxic against (HepG2) cells in highest concentration (100 µg/ml), the toxicity gradually decreased as fraction concentrations dropped, between (50-25) (µg/ml) we observed increase in viability percentage of the cells (fig. 2). After exposure to the same fraction the viability percentage of both MN1 and MDD2 cell lines significantly decreased (P ≤ 0.05) compared to the negative control between (50 and 100) concentration (µg/ml) (fig 3, 4.).

Fraction (208) shown proliferation inhibitory effect against HeLa cells as the inhibitory concentration range was between (25-12.5) (µg/ml) which is lower concentration in comparison with inhibitory concentration range determined for other cell lines exposed to the same fraction (fig 5.). Moreover, Viability percentage of both (HepG2 and MDD2) cell lines significantly decreased (P ≤ 0.05) as concentration increase from 25 (µg/ml) to 50(µg/ml) compare with negative control (fig 6, 8.).
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Discussion
Plants are a valuable source of new natural products. In spite of the availability of different methods for the discovery of novel therapeutic agents, natural products still remain one of the best candidates for new molecules (Pezzuto, 1997). Desire to develop more natural and effective anticancer drugs have encouraged investigators to explore new sources of pharmacologically active compounds, especially from natural products (Yoder et al., 2007). According to literature and previous studies Asteraeae family has the potential to show cytotoxic effect and anti-inflammatory effects toward cancer cell lines (Kasim et al., 2011). In this study two chloroformic fractions from Asteraeae family has shown antiproliferative effect on a number of cancer cell lines (HEpG2, HeLa, MN1 and MDD2).

After exposure of fraction number (207) it was observed that the antiproliferative concentration for (HepG2, MN1 and MDD2) was between (50-25) (µg/ml), in contrast same fraction has shown less toxicity toward (HeLa) cell line since significant inhibitory concentration was demonstrated in higher concentrations (100-50) (µg/ml). Moreover, fraction number 208 has shown antiproliferative effect on HeLa cell line as inhibitory concentration was between (25-12.5) (µg/ml) while the same fraction demonstrate significant antiproliferative effect for (HepG2, MN1 and MDD2) as concentration increased from 50 (µg/ml) to 25 (µg/ml).

A moderate antiproliferative effect of fraction number (208) on HeLa cell line has Shown in our present study. This fraction can be considered for further investigation as a new alternative chemotherapeutic agent for human cervical cancer.

In conclusion, results are offering that both fractions induce antiproliferative effect, so further investigations are needed in order to know the exact molecular mechanism of effect of these fractions. Therefore, optimization of the fractions concentration used in MTT assay is required in order to get the exact IC$_{50}$ value. Moreover, mechanism of action of these extracts can be considered as possible area for future studies.
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References


Samarakoon, SR., Thabrew, I., Galhena, PB., De Silva, D. and Tennekoon, KH. (2010). A comparison of the cytotoxic potential of standardized aqueous and ethanolic extracts of a polyherbal mixture comprised of Nigella sativa (seeds), Hemidesmus indicus (Roots) and Smilax glabra (rhizome). Pharmacognosy Res. 6, 335-42.
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**Tables**

Table 1: Cytotoxicity of chloroformic fractions of plants from Astraceae family in HepG2, HeLa, MN1 and MDD2 cells.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>HepG2</th>
<th>HeLa</th>
<th>MN1</th>
<th>MDD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>207</td>
<td>50-25(µg/ml)</td>
<td>100-50(µg/ml)</td>
<td>50-25(µg/ml)</td>
<td>50-25(µg/ml)</td>
</tr>
<tr>
<td>208</td>
<td>50-25(µg/ml)</td>
<td>25-12.5(µg/ml)</td>
<td>50-25(µg/ml)</td>
<td>50-25(µg/ml)</td>
</tr>
</tbody>
</table>
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Figure legends

In all figures (X) axis is showing concentrations from lowest to highest. (Y) Axis presenting viability percentages regarding to different concentrations.

Figure 1. Effects of fraction (Ap 207 FoL) on the proliferation of HeLa cells. Cells were plated into 96-well dishes (50000 cells/well) 24 hours prior to the addition of the fraction. Following 72 hours incubation with the fraction, cell proliferation was determined by the MTT assay. Each data bar represents mean ± standard deviation based on triplicate determinations in one independent experiment. The significance level observed is *p < 0.05 in comparison with the negative control group.

Figure 2. Effects of fraction (Ap 207 FoL) on the proliferation of HepG2 cells. Cells were plated into 96-well dishes (50000 cells/well) 24 hours prior to the addition of the fraction. Following 72 hours incubation with the fraction, cell proliferation was determined by the MTT assay. Each data bar represents mean ± standard deviation based on triplicate determinations in one independent experiment. The significance level observed is *p < 0.05 in comparison with the negative control group.

Figure 3. Effects of fraction (Ap 207 FoL) on the proliferation of MN1 cells. Cells were plated into 96-well dishes (50000 cells/well) 24 hours prior to the addition of the fraction. Following 72 hours incubation with the fraction, cell proliferation was determined by the MTT assay. Each data bar represents mean ± standard deviation based on triplicate determinations in one independent experiment. The significance level observed is *p < 0.05 in comparison with the negative control group.

Figure 4. Effect of fraction (Ap 207 FoL) on the proliferation of MDD2 cells. Cells were plated into 96-well dishes (50000 cells/well) 24 hours prior to the addition of the fraction. Following 72 hours incubation with the fraction, cell proliferation was determined by the MTT assay. Each data bar represents mean ± standard deviation based on triplicate determinations in one independent experiment. The significance level observed is *p < 0.05 in comparison with the negative control group.
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Figure 5. Effects of fraction (Ap 208 FS) on the proliferation of HeLa cells. Cells were plated into 96-well dishes (50000 cells/well) 24 hours prior to the addition of the fraction. Following 72 hours incubation with the fraction, cell proliferation was determined by the MTT assay. Each data bar represents mean ± standard deviation based on triplicate determinations in one independent experiment. The significance level observed is *p < 0.05 in comparison with the negative control group.

Figure 6. Effects of fraction (Ap 208 FS) on the proliferation of HepG2 cells. Cells were plated into 96-well dishes (50000 cells/well) 24 hours prior to the addition of the fraction. Following 72 hours incubation with the fraction, cell proliferation was determined by the MTT assay. Each data bar represents mean ± standard deviation based on triplicate determinations in one independent experiment. The significance level observed is *p < 0.05 in comparison with the negative control group.

Figure 7. Effects of fraction (Ap 208 FS) on the proliferation of MN1 cells. Cells were plated into 96-well dishes (50000 cells/well) 24 hours prior to the addition of the fraction. Following 72 hours incubation with the fraction, cell proliferation was determined by the MTT assay. Each data bar represents mean ± standard deviation based on triplicate determinations in one independent experiment. The significance level observed is *p < 0.05 in comparison with the negative control group.

Figure 8. Effects of fraction (Ap 208 FS) on the proliferation of MDD2 cells. Cells were plated into 96-well dishes (50000 cells/well) 24 hours prior to the addition of the fraction. Following 72 hours incubation with the fraction, cell proliferation was determined by the MTT assay. Each data bar represents mean ± standard deviation based on triplicate determinations in one independent experiment. The significance level observed is *p < 0.05 in comparison with the negative control group.
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Fig. 1

Hela 207
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Fig. 2

HepG2 207
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Fig. 3

MN1 207
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Fig. 4

MDD2 207

![Graph showing viability percentage vs. concentration (mg/ml)]
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Fig. 5

Hela 208
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Fig. 6

HepG2 208

![Graph showing viability percentage against concentration (mg/ml)]
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Fig. 7

MN1 208

![Graph showing viability % against concentration (mg/ml) for MN1 208 cancer cell line. The graph displays bars for different concentrations, with error bars indicating variability. The x-axis represents concentration (mg/ml) ranging from 0.001 to 15.000, and the y-axis shows viability % ranging from 0 to 150. Positive control bars are indicated for 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 mg/ml, with viability decreasing as concentration increases. The graph shows significant cytotoxic activity at the highest concentration.]
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Fig. 8

MDD2 208