



The Cytotoxic Effects of *Ferula Persica* var. *Persica* and *Ferula Hezarlalezarica* against HepG2, A549, HT29, MCF7 and MDBK Cell Lines

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Abstract

Cancers belong to a group of disorders which are very important for researchers. Because they have several types and cause mortality in human beings. Many investigations are performing in order to introduce cheaper drugs with lower side effects especially with natural sources. *Ferula* genus grows all over the world but some of them are endemic to Iran. Many investigations have proved different biological activity of these plants. It has been established that some *Ferula* species have cytotoxic activity. In the present study, cytotoxic effects of *F. persica* var. *persica* and *F. hezarlalezarica* which are endemic to Iran have been evaluated against tumor cell lines MCF7, HepG2, HT29, A549, and a normal cell line MDBK using MTT method. Total extracts of the plants aerial parts were prepared by 80% methanol and maceration method. Different fractions of the plants were obtained using hexane, chloroform, ethyl acetate, pure or 50% methanol. Total extracts and different fractions were used for MTT assay. The results showed that among the examined samples, only hexane and chloroform fractions of the plants had cytotoxic effects up to concentration of 100 µg/ml. So that, extracts of *F. persica* var. *persica* were more cytotoxic than *F. hezarlalezarica* (IC₅₀s, 22.3-71.8 µg/ml for *F. persica* var. *persica* and 76.7-105.3 µg/ml for *F. hezarlalezarica*). It seems that both plants are suitable for further investigations in cancer researches.

Keywords: Cytotoxicity; *Ferula hezarlalezarica*; *Ferula persica* var. *persica*.

Received: November 10, 2011; **Accepted:** January 21, 2012.

1. Introduction

Cancers belong to a group of disorders with difficulty in treatment and sometimes are incurable. Nowadays, considerable scientific and commercial interests have been

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increased for discovering new anticancer agents with natural sources [1-4]. Plants have been sources of the well known anticancer drugs such as camptothecin, podophyllotoxin and paclitaxel [5]. The potential of natural products as anticancer agents was recognized for the first time in the 1950s by the U.S. National Cancer Institute (NCI), and after that many investigations have been performed to the discovery of new natural anticancer agents [6]. Different methods are used for screening of anticancer agents. One of the techniques is MTT [3-(4,5-dimethylthiazol-2yl)-2,4-diphenyltetrazolium bromide] assay which is a simple and reliable method for preliminary evaluation of anti-cancer agents [7].

Ferula genus (Apiaceae) is one of the most important genders in Iran. These plants were used in traditional medicine as anti-convulsion, anti-swelling, anti-spasm and expectorant [8]. The genus has been represented in Iran by 31 species of which 16 are endemic to Iran [9]. Many investigations have proved different biological activity of these plants [10]. It has been established that some *Ferula* species have cytotoxic activity [11, 12]. *F. persica* is one of the endemic plants to Iran [9], and some investigations have been carried out to isolate, purify and elucidate the structure of its compounds [13-15]. But, there is no report on cytotoxic effects of the plant on MDBK, A549, HT29, HepG2 and MCF7 cell lines. *F. hezarlalehzarica* has

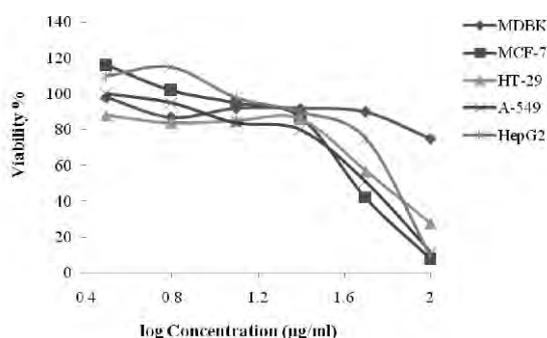


Figure 1. Viability (%) of tested cell lines against different concentrations of *F. persica* var. *persica* hexane fraction.

been identified for the first time in 2008 from Iran [16] and no phytochemical and biological study has been done on the plant so far. In this investigation, cytotoxic activity of *F. persica* var. *persica* and *F. hezarlalehzarica* aerial parts on some cell lines has been determined by using MTT method.

2. Material and methods

2.1. Chemicals

Methyl Thiazol Tetrazolium (MTT) was obtained from Sigma (Germany). Methanol, dimethyl sulfoxide (DMSO) and all other organic solvents (analytical grade) were purchased from Merck (Germany).

2.2. Plant extraction and fractionation

2.2.1. Extraction

Total plant extracts were obtained by extraction of dried and milled aerial parts of the plants with 80% methanol (1:10) using maceration method for 4 days. After every 24 h, the mixture was filtered and new solvent was added to the plant powder. The combined extracts were concentrated to dryness under vacuum pressure.

2.2.2. Fractionation

Different solvents containing hexane, chloroform, ethyl acetate, 100% and 50% methanol were used for fractionation. Each solvent was used for extraction during four days as same as total extract method. After 4 days, the plant powder was dried and new

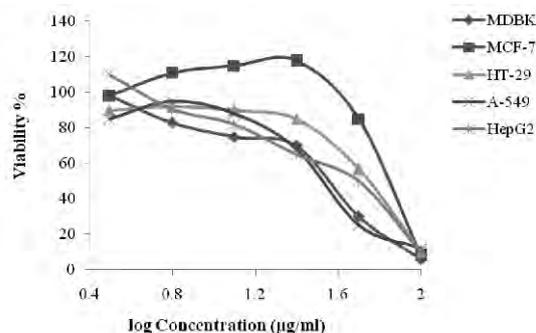


Figure 2. Viability (%) of tested cell lines against different concentrations of *F. persica* var. *persica* chloroform fraction.

solvent was added. Combined extracts of each solvent were mixed and dried.

2.3. Preparing the extracts for MTT assay

All samples were dissolved in DMSO to make the stock solution (100 µg/ml). But 50% methanolic extract of *F. hezarlalehzarica* was dissolved in water. Serial dilutions were prepared accordingly from the above stock solution to get the final concentrations (50, 25, 12.5, 6.25 and 3.125 µg/ml) with DMSO or water.

2.4. Cell cultures

HepG2 (human hepatocellular liver carcinoma), MCF7 (human breast adenocarcinoma), HT29 (human colon adenocarcinoma), A549 (human lung adenocarcinoma) and MDBK (bovine kidney cells) cell lines were obtained from Pasteur Institute of Iran. Each cell line was cultured in suitable medium for desired growth, completed with FBS (5 or 10%) in a humidified incubator at 37 °C in an atmosphere of 5% CO₂. Then the growth curve of each cell line was plotted.

2.5. MTT assay

Cytotoxic activity was assessed by MTT assay according to the method which proposed by Alley *et al.* [7] using some modification, with different concentrations of the plants extracts. The cells were seeded in 96-well plates. Three wells for each concentration

were seeded and triplicate plates were used for each cell line. Then, the cells were incubated in CO₂ incubator. After 24 h, the medium was replaced by fresh medium containing different concentrations of the plants extracts and incubated for further 72 h. The initial concentration of samples was 100 mg/ml in DMSO, which was serially diluted in the medium with two fold dilutions to give six concentrations. The medium was changed by fresh medium containing MTT with a final concentration of 0.5 mg/ml. The cells were incubated for another 4 h in a humidified atmosphere at 37 °C and after that the medium containing MTT was removed and remaining MTT-formazan crystals were dissolved in DMSO. The absorbance was measured at 570 nm using a microplate reader and viability of the cells was investigated relative to the negative control which was exposed to the solvent without extract. Tamoxifen was included as positive control.

3. Results and discussion

The results of cytotoxic assay of *F. persica* var. *persica* and *F. hezarlalehzarica* aerial parts showed that among different samples, only hexane and chloroform fractions of the plants exhibited cytotoxic effects up to concentration of 100 µg/ml (Figures 1-4), in the manner that both fractions of *F. persica* var. *persica* were more cytotoxic than *F. hezarlalehzarica* fractions against MCF7,

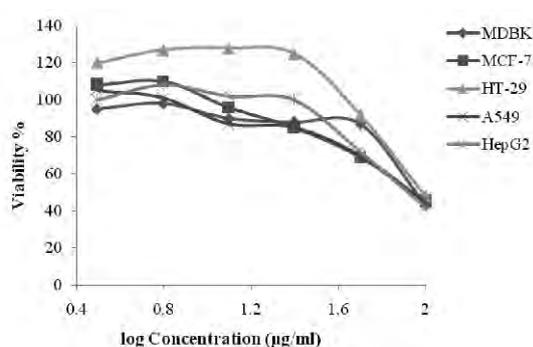


Figure 3. Viability (%) of tested cell lines against different concentrations of *F. hezarlalehzarica* hexane fraction.

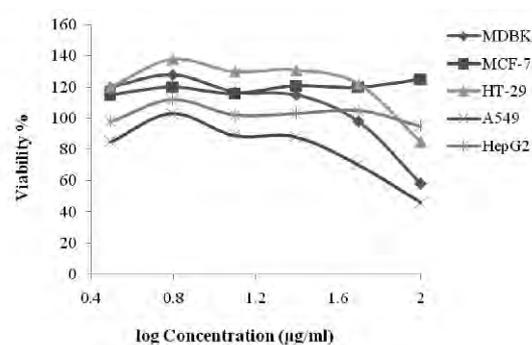


Figure 4. Viability (%) of tested cell lines against different concentrations of *F. hezarlalehzarica* chloroform fraction.

HepG2, MDBK, A549 and HT29 cell lines, except that hexane fraction of *F. persica* var. *persica*, which showed no cytotoxicity against MDBK cells up to concentration of 100 µg/ml (Figures 5 and 6). The highest cytotoxicity was observed in MDBK cell line by chloroform fraction of *F. persica* var. *persica* (IC₅₀, 22.3 µg/ml). In order to consider a compound as cytotoxic agent in treatment of cancers, it is best to have no cytotoxicity on normal cell lines such as MDBK. Regarding cytotoxicity of the chloroform fraction of *F. persica* var. *persica* and hexane fraction of *F. hezarlalehzarica* on MDBK cell line, it seems that they are not the first choices for further evaluations in cancer researches. Of course, it is possible to purify a compound without cytotoxicity on MDBK but with cytotoxic effect on other cell lines. The results also demonstrated that *F. hezarlalehzarica* chloroform extract is toxic only against A549 cell line (IC₅₀, 105.3 µg/ml which obtained from extrapolation), therefore, it is a suitable choice for more investigations in lung cancer.

Lack of cytotoxicity of the total extracts of the plants and cytotoxicity of the non-polar fractions (hexane and chloroform fractions) shows that in order to evaluate of the plant for further experiments, fractionation is necessary.

Several investigations demonstrated the cytotoxic activity of *Ferula* species. Bagheri et al. [8] determined the cytotoxicity of some *Ferula* species on *Artemia salina* as a model

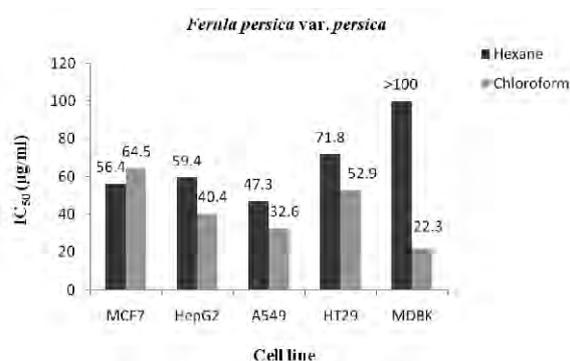


Figure 5. The IC₅₀s of hexane and chloroform fractions of *Ferula persica* var. *persica*

for evaluating general cytotoxicity. They proved that methanolic extract of *F. diversivittata*, *F. persica*, *F. ovina*, *F. badrakema*, *F. latisecta* and oleo gum resin of *F. assa-foetida* were cytotoxic with LC₅₀ values in the range of 6-321 µg/ml. Another study on *F. assa-foetida* showed that some compounds were toxic against HepG2, Hep3B and MCF7 cancer cell lines [12]. *Ferula* species are rich in coumarin compounds. Many biological activity of *Ferula* genus such as antibacterial, antiviral, cytotoxicity and anti-inflammatory effects have been attributed to sesquiterpene coumarins [10]. Iranshahi et al. have studied cancer chemoprotective activity of some terpenoid coumarins purified from *Ferula* species through inhibition of Epstein - Barr virus early antigen (EBV-EA) activation in Raji cells. Auraptene (7-gerany loxycoumarin) and umbelliprenin (7-farnesy loxycoumarin) were found as anti-tumor-promoting agents. They proposed that prenyl moiety in terpenoid coumarins plays an important role in anti-tumor promoting activity as previously reported for flavonoids, coumarins, phenylpropanoids and xanthenes [17]. An investigation on farnesiferol A (from *F. persica*) and galbanic acid (from *F. szowitsiana*) with sesquiterpene coumarin structure showed that they could inhibit P-glycoprotein-mediated rhodamine efflux in a doxorubicin resistant breast cancer cell line (MCF7/Adr). These two compounds were considered for further studies on the reversal

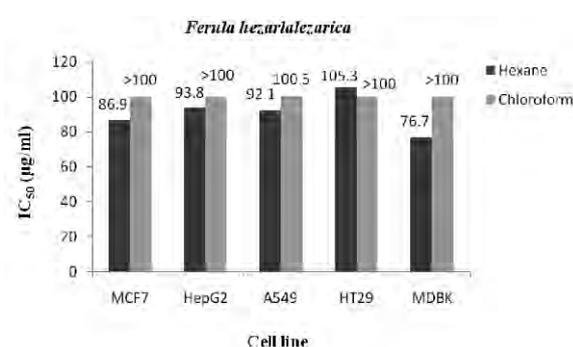


Figure 6. The IC₅₀s of hexane and chloroform fractions of *Ferula hezarlalehzarica*

of multi-drug resistance phenotype in chemotherapy of cancer patients [18]. Regarding the reported cytotoxicity of terpenoid coumarins and similarity of their structures in different *Ferula* species, these compounds may be considered as cytotoxic agents in *F. persica* var. *persica* and *F. hezarlalezarica* as well, but further studies are necessary to establish this idea.

Acknowledgment

The authors wish to thank Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences for supporting the project (No. 121) and Mr. H. Moazzeni and Mrs. A. Pirani for collection and identification of the plants. This manuscript has been obtained from Pharm. D. student thesis.

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