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IN VITRO EVALUATION FOR CYTOTOXIC ACTIVITY OF THREE *FERULA* SPECIES

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ABSTRACT: To study the effect of herbal materials as anti-cancer agents, the first step is to evaluate their cytotoxic activity; thus in the present study, the cytotoxic activity of the extracts and fractions of *Ferula szowitsiana*, *F. hirtella* and *F. oopoda* in MCF-7, HT-29, A-549 and HepG-2 cells have been investigated through MTT assay. The result showed that the *n*-hexane and chloroform fractions of *F. szowitsiana* and *F. hirtella* were the most cytotoxic to all cell lines while *F. hirtella* extracts/fractions demonstrated little or no cytotoxic activity. The results suggest that the non-polar/semi polar constituents might be the cause for the cytotoxic behavior of the species.

INTRODUCTION: Nowadays, there is a growing consideration in the use of natural materials for overcoming cancer and the development of safer and more productive therapies [1]; thus the trend towards natural materials for finding new promising agents is growing in toxicology laboratories where primary assays consist of *in vitro* evaluation of cytotoxixty to cancerous cells.

The genus *Ferula* (Apiaceae) has a wide distribution throughout Mediterranean and Middle East area, especially in countries such as Iran [2] where thirty perennial species of the genus grow. They are mostly found in mountainous regions and some are distributed in desert areas [3].

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These plants have proved to possess biological activity in different studies [4] and some *Ferula* species have demonstrated cytotoxic activity [5].

F. szowitsiana DC, *F. hirtella* Boiss and *F. oopoda* Boiss are three species with little or no history of previous cytotoxic investigations. In the present study the above *Ferula* species were collected from different reigns of Iran and the cytotoxicity of their total extract and the *n*-hexane, chloroform and methanol fraction have been evaluated against MCF-7, HepG-2, MDBK, A-549 and HT-29 cell lines.

MATERIAL AND METHODS:

Chemicals and Reagents: RPMI 1640 medium, penicillin-streptomycin, 3-(4, 5-dimethylthiazol-2-yl) -2, 4-diphenyltetrazolium bromide [MTT] (Sigma), DMEM medium and FBS (Gibco) were used in cell culture assays; all organic solvents (analytical grade) were provided from Merck.

Cell lines: The cell lines were provided from Pasteur Institute, Tehran, Iran: MCF-7 (human breast adenocarcinoma), HepG-2 (human hepatocellular carcinoma, A-549 (non-small cell lung carcinoma), HT-29 (human colorectal adenocarcinoma).

MCF-7 and HT-29 cells were cultured in DMEM medium with 5% and 10% FBS, respectively, while the other cell lines were maintained in RPMI 1640 medium with 10% FBS to obtain the desired growth. All cell lines were treated with 1% penicillin-streptomycin, in a humidified incubator at 37°C in an atmosphere of 5% CO_2.

Plant material: Whole plant of *F. szowitsiana*, aerial parts of *F. hirtella* and whole plant of *F. oopoda* were collected from Semnan, Tehran and Golestan province, respectively. The species were authenticated by Mr. Abdolbaset Ghorbani (botanist), Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. A voucher specimen of each species is deposited at TMRC herbarium for future reference. The collected parts were dried in shade and ground prior to extraction.

Extraction: 10 g of dried powder of each species was macerated with methanol 80% at room temperature for 3 days. Each day the solvent was replaced with fresh one. The combined filtrate was concentrated and further used in MTT assay.

Fractionation: 20 g of dried powder of the species was macerated with *n*-hexane at room temperature for 3 days. Each day the solvent was replaced with fresh one. After the third day the residue of the plant was extracted with chloroform and the same process continued for 3 days and also the same for methanol. The condensate fractions were used in MTT assay.

Preparation for MTT assay: The extracts/fractions were dissolved in DMSO (30 mg/mL). The final concentrations (300 µg/mL, 150 µg/mL, 75 µg/mL, 37.5 µg/mL, 18.75 µg/mL and 9.37 µg/mL) were provided from the above solution (DMSO 1%). A stock solution of extracts/fractions (10 mg/mL) in DMSO was prepared whenever they did not dissolve at higher amounts and two fold concentrations were provided from the mentioned stock solution accordingly.

MTT assay: 8000 MCF-7 cells, 15000 HepG-2 cells, 8000 A-549 cells and 5000 HT-29 cells/well were seeded in 96-well plates and the growth inhibitory effect was assessed in a micro culture tetrazolium/formazan assay (MTT assay) [6, 7]. After 24 h of incubation of cells at 37°C, the medium was replaced with fresh medium containing different concentrations of the extracts. The cells were exposed to each sample for 72 h at 37°C, thereafter the medium was replaced with fresh medium containing MTT (final concentration of 0.5 mg/mL).

The cells were incubated for another 4 h in a humidified atmosphere at 37°C. At the end of the incubation period, the medium containing MTT was removed and the remaining formazan crystals were dissolved in DMSO. The absorbance was recorded at 570 nm with an ELISA reader (TECAN). Tamoxifen was used as positive control.

The relative cell viability related to control (wells containing cells, cell culture medium and DMSO 1%) was calculated by [A]s /[A]c × 100. Where [A]s is the absorbance of the test sample and [A]c is the absorbance of control wells. To calculate IC_{50} viability versus concentrations was graphed by Microsoft Excel program [8].

RESULTS: The cytotoxic activity of the three *Ferula* species was assessed through MTT colorimetric assay. The results are demonstrated in figures 1-3. Twelve samples (extracts and fractions) were evaluated for cytotoxic activity. The IC_{50} values above 300 µg/mL were considered as inactive.

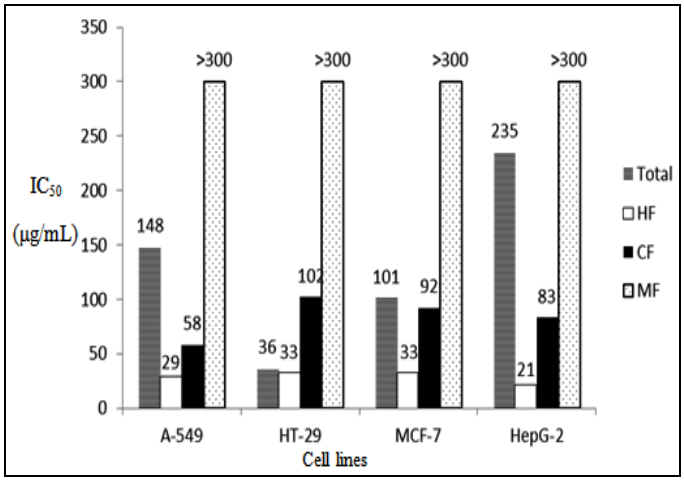

FIGURE 1: THE IC_{50} VALUES OF *FERAULA SZOWITSIANA* TOTAL EXTRACTS/FRACTIONS AGAINST A-549, HT-29, MCF-7 AND HepG2 cells: *n*-hexane fraction (HF), chloroform fraction (CF), and methanol fraction (MF)

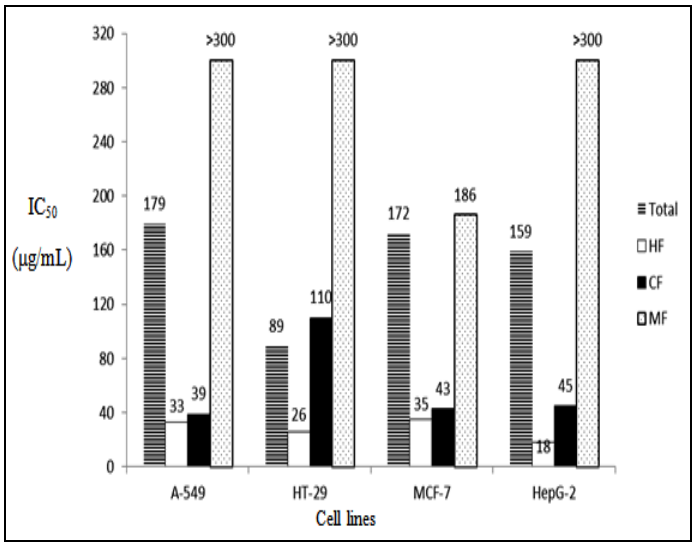

FIGURE 2: THE IC_{50} VALUES OF *FERAULA HIRTELLA* TOTAL EXTRACTS/FRACTIONS AGAINST A-549, HT-29, MCF-7 AND HEPG2 CELLS: *n*-hexane fraction (HF), chloroform fraction (CF), and methanol fraction (MF)

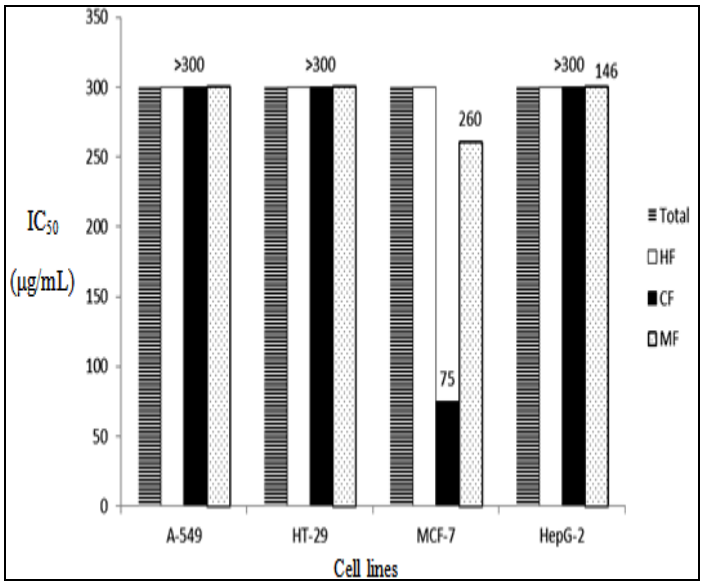

FIGURE 3: THE IC_{50} VALUES OF *FERAULA OOPODA* TOTAL EXTRACTS/FRACTIONS AGAINST A-549, HT-29, MCF-7 AND HEPG2 CELLS: *n*-hexane fraction (HF), chloroform fraction (CF), and methanol fraction (MF)

Comparing the IC_{50} values revealed that *F. szowitsiana* and *F. hirtella* total extracts demonstrated the most cytotoxic activity in HT-29 cells, also the *n*-hexane and chloroform fractions showed more cytotoxicity to all cell lines in comparison to the methanol fractions which might be implying that non-polar to semi polar compounds could be responsible for the cytotoxic activity. *Ferula oopoda* extracts and fractions exhibited less cytotoxic activity compared to the two other species and among its extracts and fraction, only the chloroform one demonstrated cytotoxic activity to MCF-7 cells.

Previous studies have shown that the methanol extract of *F. oopoda* was cytotoxic to MCF-7 cells with the IC_{50} value of 45 µg/mL [9]. No cytotoxic activity for the total extract was recorded in the present study and this could be due to the more polar nature of our extract. Also *F. szowitsiana* methanol extract has demonstrated cytotoxic activity to MCF-7 and HepG-2 cells with the IC_{50} value of 29 and 40.6 µg/mL, respectively [10]. This is also the same here, where our IC_{50} for the total extract which was obtained by methanol 80%, was higher to the both cell lines which again imply that the less polar compounds might be involved in the cytotoxic property of the species.

Phytochemical studies have resulted in isolation of cytotoxic sesquiterpenoid derivatives from the genus *Ferula* [4, 11, 12] and it is clear that most sesquiterpenoids could be partitioned in *n*-hexane and chloroform, but more phytochemical investigations and isolation of the constituents of the three species of our study, will be of great help for deeper understanding of the cytotoxic behavior in the evaluated species.

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