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Original article

# Investigating the cytotoxic effect of some medicinal plants from northern parts of Iran

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## Abstract

**Background and objectives:** Iran owns a rich and prestigious heritage of medicinal herbs but the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies. In the present study some species form northern parts of Iran were evaluated for cytotoxicity. **Methods:** Sixteen medicinal plants were extracted with methanol and screened for their cytotoxic activities. The inhibition of cell growth for these extracts was evaluated against MCF-7, WEHI-164, HepG-2 and MDBK cell lines. Their 50% inhibitions of growth ($IC_{50}$) were determined by MTT assay. Moreover, cytotoxic evaluation of different fractions of the most potent species was performed. **Results:** Among examined samples, the $IC_{50}$ values of methanol extract of *Centaurea bruguierana* (DC.) Hand.-Mzt. on mentioned cell lines were found 47.30-87.40 µg/mL. In addition, the chloroform fraction of the species was cytotoxic with $IC_{50}$ values 17.00-23.03 µg/mL. **Conclusion:** It was concluded that the chloroform fraction of *C. bruguierana* was the best candidate for identification and isolation of active principles with cytotoxic effects. These results recommend further studies about this species.

**Keywords:** *Centaurea bruguierana*, cytotoxic effect, medicinal plants, MTT, Iranian Traditional Medicine

## Introduction

Natural products and related drugs are used to treat 87% of all categorized human diseases, including bacterial infections, cancers and immunological disorders [1]. So far, it has been estimated that only 5000 plant species have been properly studied for possible medical applications [2]. Furthermore, approximately 25% of prescribed drugs in the world originate from plants and over 3000 species of plants have been reported to have anticancer properties [3,4].

Plants continue to be a rich source of therapeutic agents. The active principles of many drugs are

 
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found in plants and are produced as secondary metabolites [5]. Considering that there are 250,000 to 300,000 plant species on the planet, the majority of this treasure awaits retrieval from plants [2]. Additionally, isolation and identification of some potent anti-tumour compounds from medicinal plants, such as colchicine, paclitaxle and podophyllotoxin, has encouraged scientists to screen different plant species against cancer cell lines [6].

The screening of plants to investigate the cytotoxic effect and extraction with solvents of different polarity can improve the isolation of active compounds with the cytotoxic effect.

To investigate their cytotoxic effect, we carried out a screening of some medicinal plants with reported antimicrobial effects [7,8]. These plants were from northern parts of Iran.

## Experimental

*Plant material*

Aerial parts of the selected plants (table 1) were collected from northern parts of Iran and identified by a qualified botanist at Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. The voucher specimens were deposited at the Herbarium of TMRC.

*Extraction and fractionation*

The aerial parts of the selected plants were crushed. Whilst being constantly shaken, 50g of each powdered dried material was macerated in 500 mL methanol for 24 hours. The filtrates were evaporated to dryness. To investigate for cytotoxic effects, all methanol extracts of the plants were screened [9].

The petroleum ether and chloroform fractions of the selected plants which showed $IC_{50}$ less than 100µg/mL were prepared successively with maceration method [9].

*Cell lines*

The cell lines used in this study were MCF-7 (human breast adenocarcinoma), WEHI-164 (mouse fibro sarcoma), HepG-2 (human hepatocellular liver carcinoma) and MDBK (Madin-Darby bovine kidney). The cell lines were purchased from the Pasteur Institute, Tehran, Iran.

*In vitro cytotoxicity assay*

The cells were seeded in 96-well micro plates at $8 \times 10^3$ cells for MCF-7 and $10^4$ cells for WEHI-164, HepG-2 and MDBK cells in triplicate. The cells were then incubated at 37 °C under 5% $CO_2$

**Table 1**. Scientific name, family, location and voucher number of medicinal plants

| No | Scientific Name | Family | Location | Voucher Number |
|---|---|---|---|---|
| 1 | *Astrodaucus orientalis* (L.) Drude | Apiaceae | Tehran | TMRC 1276 |
| 2 | *Capparis spinosa* L. | Capparidacea | Golestan | TMRC 1295 |
| 3 | *Centaurea bruguierana* (DC.) Hand. – Mzt. | Asteraceae | North Khorasan | TMRC 1291 |
| 4 | *Cephalanthera caucasica* kränzl | Orchidaceae | Mazandaran | TMRC 1288 |
| 5 | *Colutea persica* Boiss. | Fabaceae | Mazandaran | TMRC 1280 |
| 6 | *Erodium oxyrrhynchum* M.B. | Geraniaceae | Tehran | TMRC 1277 |
| 7 | *Ficus carica* L. *var. genuina* Boiss. | Moraceae | Tehran | TMRC 1212 |
| 8 | *Heracleum persicum* Desf. Ex. Fischer | Apiaceae | Tehran | TMRC 692 |
| 9 | *Linaria pyramidata* Lam. | Scrophulariaceae | North Khorasan | TMRC 1293 |
| 10 | *Marrubium vulgare* L. | Lamiaceae | Mazandaran | TMRC 1286 |
| 11 | *Minuartia lineata* Bornm. | Caryophyllaceae | Mazandaran | TMRC 1279 |
| 12 | *Paliurus spina-christi* Mill. | Rhamnaceae | Mazandaran | TMRC 1282 |
| 13 | *Papaver bracteatum* Lindl. | Papaveraceae | Ardabil | TMRC 1297 |
| 14 | *Pteropyrum aucheri* Jaub. & Spach | Polygonaceae | Tehran | TMRC 1285 |
| 15 | *Roemeria refracta* DC. | Papaveraceae | North Khorasan | TMRC 1292 |
| 16 | *Tamarix aralensis* Bge. | Tamaricaceae | Gilan | TMRC 1284 |

atmosphere. After 24 h, the medium was replaced with a fresh one, containing different concentrations of samples to be tested.

The initial concentration of samples was 100 µg/mL in dimetylsulfoxide (DMSO), which was serially prepared with two fold dilutions to make six concentrations.

The cells were exposed to each sample for 72 h at 37 °C. The cytotoxicity of the plant extracts were determined by a rapid colorimetric method (MTT assay). They were then compared to the untreated controls [10]. The absorbance was recorded at 570 nm by an ELISA plate reader. To calculate $IC_{50}$, viability (%) versus log concentrations was graphed by Microsoft Excel program. Tamoxifen was used as the positive control.

**Results and Discussion**

In our study, the cytotoxic effect of 16 medicinal plant methanol extracts against four cell lines was determined using the MTT assay. The cytotoxic results are shown in table 2.

Previous studies have revealed the *in vitro* cytotoxic effect of fruit and leaf extracts, as well as the latex of *Ficus carica* against HeLa cell line determined by MTT assay. The results have indicated that the latex and different extracts of *Ficus carica* could reduce the viability of the HeLa cells at concentrations as low as 2µg/mL in dose dependent manner [11]. The potential of anticancer activity of *Astrodaucus orientalis* extracts determined with MTT assay has been reported in human breast carcinoma cell line, T-47D [12]. In other studies, cytotoxic activity of the essential oils of *Heracleum persicum* has been investigated showing cytotoxic effects in brine shrimp lethality assay [13].

According to table 2, except for *Centaurea bruguierana*, other methanol extracts did not show cytotoxicity against the cell lines.

It could be argued that the above mentioned plants might have shown selective *in vitro* cytotoxicity against some cell lines and they were not toxic to the cell lines of the present study.There is also another possibility that these speciesmight have presented their cytotoxicity by a mechanism that could not be detected by MTT assay. Thus, other methods of evaluation could be suggested for further studies.

As mentioned before, $IC_{50}$ of *Centaurea bruguierana* methanol extract was less than 100 µg/mL gainst MCF-7, WEHI-164, HepG-2 and MDBK cells. *Centaurea* is the largest genus of the family Asteraceae and represents approximately 47 species in Iran [14].

**Table 2.** $IC_{50}$ of medicinal plants against MCF-7, WEHI-164, HepG-2 and MDBK cell lines

| **Scientific name** | **($IC_{50}$ µg/mL)** | | | |
|---|---|---|---|---|
| | **MCF-7** | **WEHI-164** | **HepG-2** | **MDBK** |
| *Astrodaucus orientalis* (L.) Drude | > 100 | > 100 | > 100 | > 100 |
| *Capparis spinosa* L. | > 100 | > 100 | > 100 | > 100 |
| *Centaurea bruguierana* (DC.) Hand. – Mzt. | 47.30 | 50.66 | 84.70 | 53.27 |
| *Cephalanthera caucasica* kränzl | > 100 | > 100 | > 100 | > 100 |
| *Colutea persica* Boiss. | > 100 | > 100 | > 100 | > 100 |
| *Erodium oxyrrhnchum* M.B. | > 100 | > 100 | > 100 | > 100 |
| *Ficus carica* L. *var. genuina* Boiss. | > 100 | > 100 | > 100 | > 100 |
| *Heracleum persicum* Desf. Ex. Fischer | > 100 | > 100 | > 100 | > 100 |
| *Linaria pyramidata* Lam. | > 100 | > 100 | > 100 | > 100 |
| *Marrubium vulgare* L. | > 100 | > 100 | > 100 | > 100 |
| *Minuartia lineata* Bornm. | > 100 | > 100 | > 100 | > 100 |
| *Paliurus spina-christi* Mill. | > 100 | > 100 | > 100 | > 100 |
| *Papaver bracteatum* Lindl | > 100 | > 100 | > 100 | > 100 |
| *Pteropyrum aucheri* Jaub. & Spach | > 100 | > 100 | > 100 | > 100 |
| *Roemeria refracta* DC. | > 100 | > 100 | > 100 | > 100 |
| *Tamarix aralensis* Bge. | > 100 | > 100 | > 100 | > 100 |
| Tamoxifen | 3.69 | 19.1 | 4.38 | 4.39 |

Many of *Centaurea* species were used as anti-diabetic, anti-rheumatic and anti-inflammatory agents, based on Iranian Traditional Medicine manuscripts [15] and ethnobotanical knowledge [16]. They were additionally used for their anti-bacterial and cytotoxic effects [17].

These species have been the subject of many investigations which has led to the isolation of various types of compounds. For example, sesquiterpene lactones, flavonoids and lignans [17]. There has been a report about investigation of *C. bruguierana* cytotoxicity by MTT assay. The data were collected from southern parts of Iran, against HT-29 (colon carcinoma), Caco-2 (colon adenocarcinoma), T-47D (breast ductal carcinoma) and NIH-3T3 (Swiss embryo fibroblast) cell lines. The results had demonstrated cytotoxic activity against Caco-2 cell line with an $IC_{50}$ value of 10 µg/mL [18].

In our study *C. bruguierana* was collected from northern parts of Iran and its methanol extract demonstrated $IC_{50}$ from 47.30 to 84.70µg/mL.

The aim of our study was to acquire the most cytotoxic fraction of the selected plant; thus, *C. bruguierana* was our candidate for further fractionation. The cytotoxic results of its fractions are shown in table 3.

**Table 3.** $IC_{50}$ of fractions of *Centaurea bruguierana* against cell lines

| Extract | ($IC_{50}$ µg/mL) | | | |
|---|---|---|---|---|
| | MCF-7 | WEHI-164 | HepG-2 | MDBK |
| Petroleum ether | > 100 | > 100 | > 100 | > 100 |
| Chloroform | 23.03 | 14.44 | 21.10 | 17.00 |

These results are in accordance with previous studies [18].

According to table 3, the chloroform fraction of *C. bruguierana* showed the lowest $IC_{50}$ against selected cell lines, in comparison with other fractions ($IC_{50}$ ranging from 14.44 to 23.03 µg/mL). Therefore the chloroform fraction of *C. bruguierana* is the best candidate for isolation and identification of active principles with cytotoxic effects as well as investigating their mechanism of inhibition.

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**Declaration of interest**

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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