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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 from 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<sub>50</sub>) were determined by MTT assay. Moreover, cytotoxic evaluation of different fractions of the most potent species was performed. **Results:** Among examined samples, the IC<sub>50</sub> 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<sub>50</sub> 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

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<sub>50</sub> 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<sub>2</sub>

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

No	Scientific Name	Family	Location	Voucher Number
1	<i>Astrodaucus orientalis</i> (L.) Drude	Apiaceae	Tehran	TMRC 1276
2	<i>Capparis spinosa</i> L.	Capparidaceae	Golestan	TMRC 1295
3	<i>Centaurea bruguierana</i> (DC.) Hand. – Mzt.	Asteraceae	North Khorasan	TMRC 1291
4	<i>Cephalanthera caucasica</i> kränzl	Orchidaceae	Mazandaran	TMRC 1288
5	<i>Colutea persica</i> Boiss.	Fabaceae	Mazandaran	TMRC 1280
6	<i>Erodium oxycorymbum</i> M.B.	Geraniaceae	Tehran	TMRC 1277
7	<i>Ficus carica</i> L. var. <i>genuina</i> Boiss.	Moraceae	Tehran	TMRC 1212
8	<i>Heracleum persicum</i> Desf. Ex. Fischer	Apiaceae	Tehran	TMRC 692
9	<i>Linaria pyramidata</i> Lam.	Scrophulariaceae	North Khorasan	TMRC 1293
10	<i>Marrubium vulgare</i> L.	Lamiaceae	Mazandaran	TMRC 1286
11	<i>Minuartia lineata</i> Bornm.	Caryophyllaceae	Mazandaran	TMRC 1279
12	<i>Paliurus spina-christi</i> Mill.	Rhamnaceae	Mazandaran	TMRC 1282
13	<i>Papaver bracteatum</i> Lindl.	Papaveraceae	Ardabil	TMRC 1297
14	<i>Pteropyrum aucheri</i> Jaub. & Spach	Polygonaceae	Tehran	TMRC 1285
15	<i>Roemeria refracta</i> DC.	Papaveraceae	North Khorasan	TMRC 1292
16	<i>Tamarix aralensis</i> 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 dimethylsulfoxide (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<sub>50</sub>, 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 species might 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<sub>50</sub> of *Centaurea bruguierana* methanol extract was less than 100 µg/mL against 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<sub>50</sub> of medicinal plants against MCF-7, WEHI-164, HepG-2 and MDBK cell lines (IC<sub>50</sub> µg/mL)

Scientific name	IC <sub>50</sub> µg/mL			
	MCF-7	WEHI-164	HepG-2	MDBK
<i>Astrodaucus orientalis</i> (L.) Drude	> 100	> 100	> 100	> 100
<i>Capparis spinosa</i> L.	> 100	> 100	> 100	> 100
<i>Centaurea bruguierana</i> (DC.) Hand. – Mzt.	47.30	50.66	84.70	53.27
<i>Cephalanthera caucasica</i> Kränzl	> 100	> 100	> 100	> 100
<i>Colutea persica</i> Boiss.	> 100	> 100	> 100	> 100
<i>Erodium oxycanthum</i> M.B.	> 100	> 100	> 100	> 100
<i>Ficus carica</i> L. var. <i>genuina</i> Boiss.	> 100	> 100	> 100	> 100
<i>Heracleum persicum</i> Desf. Ex. Fischer	> 100	> 100	> 100	> 100
<i>Linaria pyramidata</i> Lam.	> 100	> 100	> 100	> 100
<i>Marrubium vulgare</i> L.	> 100	> 100	> 100	> 100
<i>Minuartia lineata</i> Bornm.	> 100	> 100	> 100	> 100
<i>Paliurus spina-christi</i> Mill.	> 100	> 100	> 100	> 100
<i>Papaver bracteatum</i> Lindl.	> 100	> 100	> 100	> 100
<i>Pteropyrum aucheri</i> Jaub. & Spach	> 100	> 100	> 100	> 100
<i>Roemeria refracta</i> DC.	> 100	> 100	> 100	> 100
<i>Tamarix aralensis</i> 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<sub>50</sub> value of 10 µg/mL [18].

In our study *C. bruguierana* was collected from northern parts of Iran and its methanol extract demonstrated IC<sub>50</sub> from 47.30 to 84.70 µg/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<sub>50</sub> of fractions of *Centaurea bruguierana* against cell lines

Extract	(IC <sub>50</sub> 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<sub>50</sub> against selected cell lines, in comparison with other fractions (IC<sub>50</sub> 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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