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Original article

Cytotoxic activity of some ethnic medicinal plants from southwest of Iran

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Abstract

Background and objectives: Many people in ethnic groups of the world have trusted in plants for disease management and cure. Medicinal plants have always played a great role in the lives of Iranian people in the past and present and with no doubt in the future. Healers in different regions of Iran have been using medicinal herbs and one rich source for these cures is located in south-west of Iran, Kohgiluyeh va Boyer Ahmad province. Some species from this province have been selected for the present study and their cytotoxic activity has been evaluated. **Methods:** The methanol extracts of the 26 species were obtained by maceration and the extracts were investigated for cytotoxic activity in MTT assay. **Results:** The results revealed that four out of twenty six plants were toxic to MCF-7, A-549, HepG-2 and HT-29 cells. **Conclusion:** The findings of the present study specially the species with lower IC_{50} values (*Eryngium billardieri* and *Nerium indicum*) are suggested for further investigations in cancer studies.

Keywords*: Datura innoxia*, *Eryngium billardieri*, Kohgiluyeh va Boyer Ahmad, *Nerium indicum*, *Thymus daenensis*

Introduction

Kohgiluyeh va Boyer Ahmad is located in the mountainous regions of south west of Iran [1]. The province consists of seven townships: Boyer Ahmad, Kohgiluyeh, Gachsaran, Dena, Bahmai, Basht and Charam. With an area of 16264 km^2, it connects Fars, Isfahan, Chaharmahal and Bakhtiari, Khoozestan and Booshehr Provinces. Lots of water resources, beautiful woods and sown fields, natural mines, diverse flora and animals and high mountains are characteristics of this province [2]. Since there has been a historical connection between nature and people of this province, most nomadic families of the region are aware about local medicinal plants and their healing properties. A recent ethnobotanical study has revealed that 138 plant species mostly from Asteraceae and Lamiaceae are traditionally used by local people of Kohgiluyeh va Boyer Ahmad both for medicinal and non-medicinal purposes [3]. Considering the importance of the medicinal plants of the area, in the present study, different species from Kohgiluyeh and Boyer

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Ahmad have been collected and evaluated for their possible cytotoxic properties.

Experimental

Chemicals and reagents

Methanol from Merck, RPMI-1640 medium, penicillin-streptomycin and MTT [3-(4, 5-dimethylthiazol-2-yl) -2, 4-diphenyltetrazolium bromide] from Sigma and Dulbecco's Modified Eagle's Medium (DMEM) and Fetal Bovine Serum (FBS) from Gibco, were provided for the study.

Cell lines

MCF-7 (human breast adenocarcinoma), HepG-2 (human hepatocellular carcinoma), A-549 (non-small cell lung carcinoma) and HT-29 (human colorectal adenocarcinoma) were obtained from pasture Institute, Iran. DMEM medium with 5% and 10% FBS, respectively was used for culturing MCF-7 and HT-29 cells. HepG-2 and A-549 cells were maintained in RPMI-1640 medium with 10% FBS. The cells were all treated with 1% penicillin-streptomycin, in a humidified incubator at 37 °C in an atmosphere of 5% CO_2.

Plant material

The plant materials were collected from Kohgiluyeh and Boyer Ahmad province, Iran (2009-2011) and were identified by botanists at the Traditional Medicine and Material Medica Reasearch Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. The voucher specimens of the plants were kept at the Herbarium of TMRC (table 1).

Extraction

The dried sample of the species was ground and 5 g of each was macerated with methanol for 24 h. The concentrated filtrate was kept in refrigerator for cytotoxicity assay.

Preparation of the extracts

Dried extracts were dissolved in DMSO (10 mg/mL) and serial concentrations were made accordingly by diluting with culture medium. The final concentrations were 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL and 3.125 µg/mL.

MTT assay

Viability of the cells was assessed by MTT assay [4]. MCF-7, HepG-2, A-549 and HT-29 cells were seeded at 8×10^3, 15×10^3, 8×10^3 and 5×10^3 per well, respectively in 96-well plates and incubated at 37 °C. After 24 h, the medium was replaced with fresh medium containing the extracts. After 72 h exposure of cells at 37 °C to different concentrations of the extracts, the medium was replaced with fresh medium containing MTT with a final concentration of 0.5 mg/mL. After another 4 h incubation, 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). 5-FU was used as the positive control. The relative cell viability (%) was calculated by the following equation: (A samples/A control) × 100. Where A samples was the absorbance of wells containing the extracts and A control was the absorbance of wells in absence of extracts. To calculate IC_{50}, dose–response curves were graphed by Microsoft Excel [5,6].

Results and Discussion

The results of the cytotoxicity evaluations are presented in table 1.

As shown in the table, four out of twenty six species demonstrated cytotoxic activity in MTT assay. Among the positive results, *Eryngium billardieri* and *Nerium indicum* were toxic to all tested cell lines whereas *Datura innoxia*, and *Thymus daenensis* showed cytotoxicity to (MCF-7 and HT-29) and (MCF-7, HT-29 and A-549) cells, respectively.

Previous works about the medicinal plants of our study revealed that different species of *Eryngium* have demonstrated biological activities including cytotoxic, apoptotic, anti-inflammatory, anti-snake and scorpion venoms, antibacterial, antifungal, antimalarial, antioxidant, and antihyperglycemic properties [7-10]; however, it

Table 1. The results of cytotoxicity studies of the species from Kohgiluyeh va Boyer Ahmad province

No.	Scientific Name	Herbarium No.	IC_{50} (µg/mL)			
			MCF-7	HT-29	HepG-2	A-549
1	*Alcea calverti* (Boiss.) Boiss.	TMRC-3276	-	-	-	-
2	*Astragalus fasciculifolius* Boiss.	TMRC-2057	-	-	-	-
3	*Astragalus ovinus* Boiss.	TMRC-2295	-	-	-	-
4	*Carthamus oxyacantha* M.B.	TMRC-3279	-	-	-	-
5	*Cerasus microcarpa* Boiss.	TMRC-2870	-	-	-	-
6	*Chenopodium foliosum* (Moench) Aschers.	TMRC-2243	-	-	-	-
7	*Datura innoxia* Miller	TMRC-2049	59.4	56.5	-	-
8	*Epilobium minutiflorum* Hausskn.	TMRC-2788	-	-	-	-
9	*Eremostachys adenantha* Jaub. & Spach	TMRC-2202	-	-	-	-
10	*Eryngium billardieri* F. Delaroche	TMRC-2291	6.5	6.7	59.9	37.6
11	*Ferulago angulata* (Schiletch) Boiss.	TMRC-2800	-	-	-	-
12	*Fraxinus rotundifolia* Mill.	TMRC-3277	-	-	-	-
13	*Gypsophila polyclada* Fenzl ex Boiss.	TMRC-2873	-	-	-	-
14	*Malva parviflora* L.	TMRC-3287	-	-	-	-
15	*Marrubium astracanicum* Jacq.	TMRC-2300	-	-	-	-
16	*Mindium laevigatum* (Vent.) Rech. f. & Schiman-Czeika	TMRC-3286	-	-	-	-
17	*Nasturtium officinale* (L.) R. Br.	TMRC-3297	-	-	-	-
18	*Nerium indicum* Miller	TMRC-3280	3.1	15.4	99.3	2.0
19	*Prangos uloptera* D. C.	TMRC-3294	-	-	-	-
20	*Rosa canina* L.	TMRC-2343	-	-	-	-
21	*Sanguisorba minor* Scop.	TMRC-3139	-	-	-	-
22	*Satureja bachtiarica* Bunge	TMRC-2780	-	-	-	-
23	*Teucrium polium* L.	TMRC-3292	-	-	-	-
24	*Thymus daenensis* Celak.	TMRC-3291	84.1	61.4	-	26.2
25	*Turgenia latifolia* (L.) Hoffm	TMRC-3293	-	-	-	-
26	*Verbascum songaricum* Schrenk.	TMRC-3290	-	-	-	-

seems not much work has been carried out about the cytotoxicity of *Eryngium billardieri* although it has shown prophage induction ability in *Escherichia coli* K-12(λ) which somehow demonstrates its effect on DNA [11].

As for *Nerium indicum*, compounds from the leaves of the species have shown cytotoxic activity to HeLa and A-549 cells in an *in vitro* study [12]. *Datura innoxia* extract has also been evaluated for cytotoxicity in brine shrimp and *in vitro* cell toxicity assay to show promising cytotoxic results [13]. A withanolide from this species named dinoxin B, has been found to possess cytotoxic results in an assay for evaluating viability of mammalian cancerous cells [14].

There are some reports on different biological activities such as antioxidant, immunomodulatory and antibacterial properties of *Thymus daenensis*; however, it seems sufficient information about its cytotoxicity is missing [15-18].

The present work complies with previously reported cytotoxicity of *Nerium indicum* and *Datura innoxia*, and is complementary to the data

about *Thymus daenensis*, and *Eryngium billardieri*, giving useful information about the cytotoxic potential of the species. More molecular approach and investigating the probable apoptotic mechanism of the cytotoxic activity of these four species could be continued in further studies.

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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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