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Original article

Cytotoxic activity screening of some medicinal plants from south of Iran

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Abstract

Background and objectives: Cancer is a public health problem all over the world. Herbal medicines have a vital role in the prevention and treatment of cancer and large numbers of plants and their isolated constituents have shown potential anticancer activity. **Methods**: Twenty seven medicinal plants from southern Iran provinces have been extracted with methanol and screened for their cytotoxic activity against MCF-7, WEHI-164, HepG-2, MDBK and A-549 cell lines by MTT assay. **Results**: The methanol extracts of two species, *Calotropis procera* and *Juniperus excelsa,* demonstrated to be more effective compared to other extracts. **Conclusion**: The above species are proper candidates for further cancer studies.

Keywords*:* cytotoxic activity, Iran, medicinal plants, MTT assay

Introduction

Cancer is a public health problem all over the world which may affect many different parts of the body. If the process is not controlled, it may progress until it causes the death of the organism [1]. Plants are promising sources of new bioactive compounds. The herbal medicines have a vital role in the prevention and treatment of cancer and they are also commonly accessible [2]. About 80% of the population in developing countries relies on traditional plant-based medicines for their primary health care needs. Use of ethnobotanical information in medicinal plant researches has gained considerable attention in the scientific community [3]. Natural extracts and biologically active compounds isolated from plant species used in traditional medicine could be resources for new drugs [4]. Over 50% of drugs used in clinical trials for anticancer activity have been isolated from natural sources or are related to them. Hence, the search for natural products to be used in cancer therapy represents an area of great interest in which the plant kingdom has been the most important source, providing many anti-tumor agents with novel structures and unique mechanisms of action [5].

Cytotoxicity screening models are the preliminary methods for selection of active plant extracts against cancer [6-8].

In the course of our screening studies we performed the present study to evaluate *in vitro*

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cytotoxic activity of twenty seven plant extracts that were collected from south of Iran. Our ethnobotanical studies had revealed that some of these medicinal plants have been used by healers to treat illnesses with cancer-like symptoms. The mentioned species were evaluated against MCF-7, HepG-2, A-549, WEHI-164 and MDBK cells using Methyl Thiazol Tetrazolium (MTT) assay.

Experimental

Plant material

Based on ethnobotanical knowledge, different plant parts of 27 species were collected from Sisatan-va-Baluchestan, Bushehr, Hormozgan and Fars provinces located in the south of Iran in 2010. Voucher specimens have been deposited in the Herbarium of Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. A list of family, voucher numbers, parts used and location of the investigated species has been presented in table 1

Extraction and preparation for MTT assay

Plant materials were dried at room temperature and were ground. 10 g of the powdered of each species was macerated with methanol 80% at room temperature for 3 days. The filterate was concentrated and dissolved in dimethylsulfoxide (DMSO) to prepare the stock solutions. Subsequently, all extracts were stored at 4 °C until the cytotoxic examination.

Fractionation

Different solvents including petroleum ether, chloroform and methanol were used for fractionation of the species which had shown to be cytotoxic in MTT assay with a similar method as extraction. The fractions were further tested on the same cell lines that had shown to be cytotoxic in the earlier studies.

Cell lines

The cell lines MCF-7 (human breast adenocarcinoma), HepG-2 (human hepatocellular carcinoma), WEHI-164 (mouse fibrosarcoma), A-549 (non-small cell lung carcinoma) and MDBK (Madin-Darby bovine kidney), were provided from the Pasteur Institute, Tehran, Iran. Each cell line was cultured in suitable medium to obtain the desired growth and the growth curve of each cell line was plotted.

MTT assay

The MTT assay was performed to evaluate the cytotoxicity of the selected plant extracts. The cells were seeded in 96-well plates at 6×10^3 for MCF-7, 15×10^3 for HepG-2, 8.5×10^3 for A-549 and 6×10^3 for WEHI-164 cell lines in each well. Following 24 h incubation, the cells were treated with different concentrations (the maximum concentration was 50 μg/mL) of the plant extract for 72 h. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide solution (MTT) solution (5 mg/mL of final concentration) was added to each well and they were incubated at 37 °C for 4 h. Then the supernatant was removed and the resultant formazan crystals were dissolved in DMSO. The amount of produced formazan is directly proportional to the number of living cells. The absorbance was measured using a microplate reader at 570 nm. . Tamoxifen was used as the positive control in the present study.

Results and Discussion

Identification of medicinal plants with significant cytotoxic potential useful for the development of cancer therapeutics has gained increasing importance in the last decade, and research in this field is still expanding [9].

In the present study, the cytotoxic effect of 27 methanol plant extracts against four human cancer cell lines MCF-7, HepG-2, WEHI-164, A-549 and also the normal cell line ,MDBK, was determined using the MTT assayThe results have been summarized in table 2.

The most cytotoxic activity was found in the aerial parts of *Calotropis procera* (Wild.) R. Br. and the flowering branches of *Juniperus excelsa* M. B. None of the assessed extracts exhibited considerable cytotoxicity to WEHI-164 cells in

Table1. Medicinal plants selection in south of Iran

NO.	Family	Scientific Name	Voucher Number	Parts used	Province
1	Avicenniaceae	*Avicennia marina* (Forssk.) Vierh.	TMRC1432	Young branches	Hormozgan
2	Asteraceae	*Calendula persica* C. A. Mey.	TMRC1353	Flowers and fruits	Fars
3	Asclepiadaceae	*Calotropis procera* (Willd.) R. Br.	TMRC1403	Aerial parts	Bushehr
4	Capparaceae	*Capparis cartilaginea* Decne.	TMRC1423	Flowering branches	Hormozgan
5	Boraginaceae	*Cordia myxa* L.	TMRC1379	Flowering branches	Bushehr
6	Sapindaceae	*Dodonea viscosa* (L.) Jacq.	TMRC1377	Flowering branches	Bushehr
7	Brassicaceae	*Eruca sativa* Lam.	TMRC1425	Whole plant	Hormozgan
8	Moraceae	*Ficus benghalensis* L.	TMRC1378	Fruit-bearing branches	Bushehr
9	Rutaceae	*Haplophyllum canaliculatum* Boiss.	TMRC1239	Aerial parts	Fars
10	Asteraceae	*Hertia angustifolia* (DC.) O. Kuntze	TMRC2084	Aerial parts	Hormozgan
11	Cupressaceae	*Juniperus excelsa* M. Bieb. M. Bieb.	TMRC1433	Flowering branches	Hormozgan
12	Solanaceae	*Lycium shawii* Romer & Schult	TMRC1387	Flowering branches	Bushehr
13	Resedaceae	*Ochradenus aucheri* Boiss.	TMRC1399	Flowering branches	Bushehr
14	Lamiaceae	*Otostegia michauxii* Briq.	TMRC1152	Aerial parts	Fars
15	Lamiaceae	*Otostegia persica* (Burm.) Boiss.	TMRC918	Leaves and fruits	Sistan-va-Baluchestan
16	Asclepiadaceae	*Periploca aphylla* Decne.	TMRC1372	Flowering branches	Bushehr
17	Resedaceae	*Reseda aucheri* Boiss.	TMRC1394	Whole plant	Bushehr
18	Salvadoraceae	*Salvadora persica* L.	TMRC940	Aerial parts	Sistan-va-Baluchestan
19	Salvadoraceae	*Salvadora persica* L.	TMRC1437	Young branches	Hormozgan
20	Fabaceae	*Taverniera cuneifolia* (Roth) Arn.	TMRC1444	Aerial parts	Hormozgan
21	Lamiaceae	*Teucrium polium* L.	TMRC2095	Aerial parts	Hormozgan
22	Verbenaceae	*Vitex pseudo-negundo* (Hausskn.) Hand.-Mzt.	TMRC1153	Leaves and fruits	Fars
23	Solanaceae	*Withania coagulans* (Stocks) Dun.	TMRC932	Leaves	Sistan-va-Baluchestan
24	Lamiaceae	*Zataria multiflora* Boiss.	TMRC1429	Young branches	Hormozgan
25	Lamiaceae	*Zhumeria majdae* Rech. f. & Wendelbo	TMRC1435	Whole plant	Hormozgan
26	Rhamnaceae	*Ziziphus spina-christi* (L.) Wild.	TMRC1374	Flowering branches	Bushehr
27	Zygophyllaceae	*Zygophyllum eurypterum* Boiss. & Buhse	TMRC1427	Flowering branches	Bushehr

the evaluated concentrations.

Crude methanol extract of *Calotropis procera* demonstrated cytotoxic activity against 4 cell lines: MCF-7, HepG-2, MDBK, A-549 (IC_{50} 6.05, 11.64, 12.16, 1.9 µg/mL, respectively). *Juniperus excelsa* only showed cytotoxicity on MCF-7 (IC_{50} 31.51 µg/mL). Petroleum ether, chloroform and methanol fractions of these two species have also been evaluated in MCF-7, HepG-2, A-549 and MDBK cell lines and the results have been shown in table 2. *C. procera*, which is a wild growing plant, is well known for its medicinal uses in traditional system of medicine for treatment of variety of diseases that include leprosy, ulcers, tumors and piles. These effects have been confirmed by various scientific experiments [10]. The chloroform extract of the roots has shown to possess anti-inflammatory and analgesic properties [11]. The anti-inflammatory and analgesic properties were also exhibited by the latex of the plant which is abundant in the aerial parts [12]. In 2012, the effect of the dried latex (DL) and the flowers of *C. procera* and its ethanol extract have been investigated against MCF-7 and HeLa cell lines. The ethanol extract of DL and flowers showed cytotoxic properties against both MCF-7 and HeLa cells [13]. In 2013, different extracts of *C. procera* leaves were evaluated for *in-vitro* cytotoxic activity against Hep-2 (human larynx epithelial carcinoma) cell line. The results suggested that the *n*-butanol extract had the most pronounced cytotoxicity against the Hep-2 cells [14]. In this survey, the methanol fraction of *C. procera* was cytotoxic

Table 2. Cytotoxic activity of medicinal plants from south of Iran against HepG-2, MCF-7, MDBK, WEHI, A-549 cell lines

NO.	Scientific Name	IC_{50} (µg/ mL)				
		MCF-7	HepG- 2	MDBK	WEHI-164	A-549
1	*Avicennia marina* L.	>50	>50	>50	>50	>50
2	*Calendula persica* C. A. Mey.	>50	>50	>50	>50	>50
3	*Calotropis procera* (Wild.) R. Br.	6.05	11.64	12.16	>50	1.9
4	*Calotropis procera* Ed*	>50	>50	>50	>50	>50
5	*Calotropis procera* Ch**	5.19	>50	9.21	>50	>50
6	*Calotropis procera* Met***	4.96	>50	21.42	>50	4.84
7	*Capparis cartilaginea* Decne.	>50	>50	>50	>50	>50
8	*Cordia myxa* L.	>50	>50	>50	>50	>50
9	*Dodoanea viscose (*L.) Jacp.	>50	>50	>50	>50	>50
10	*Eruca sativa* Lam.	>50	>50	>50	>50	>50
11	*Ficus benghalensis* L.	>50	>50	>50	>50	>50
12	*Haplophyllum canaliculatum* Boiss.	>50	>50	>50	>50	>50
13	*Hertia intermedia* (Boiss.) O.Kuntze.	>50	>50	>50	>50	>50
14	*Juniperus excelsa* M. B.	31.51	>50	>50	>50	>50
15	*Juniperus excelsa* Pet*	0.508	N	N	>50	N
16	*Juniperus excelsa* Ch**	2.192	N	N	>50	N
17	*Juniperus excelsa* Met***	7.52	N	N	>50	N
18	*Lycium shawii* Roemer & Schult	>50	>50	>50	>50	>50
19	*Ochradenus aucheri* Boiss.	>50	>50	>50	>50	>50
20	*Otostegia michauxii* Briq.	>50	>50	>50	>50	>50
21	*Otostegia persica (Burm.)* Boiss.	>50	>50	>50	>50	>50
22	*Periploca aphylla* Decne.	>50	>50	>50	>50	>50
23	*Reseda aucheri Boiss.*	>50	>50	>50	>50	>50
24	*Salvadora persica* L. (Aerial parts)	>50	>50	>50	>50	>50
25	*Salvadora persica* L. (Young branches)	>50	>50	>50	>50	>50
26	*Taverniera cuneifolia* (Roth) Arn.	>50	>50	>50	>50	>50
27	*Teucrium polium* L. var. *gnaphlodes* Benth.	>50	>50	>50	>50	>50
28	*Vitex negundo* L.	>50	>50	>50	>50	>50
29	*Withania coagulans* Dun.	>50	>50	>50	>50	>50
30	*Zataria multiflora* Boiss.	>50	>50	>50	>50	>50
31	*Zhumeria majdae* Rech. F&Wendelbo	>50	>50	>50	>50	>50
32	*Ziziphus spina- christi* (L.) Willd.	>50	>50	>50	>50	>50
33	*Zygophyllum eurypetrum* Boiss. & Buhse	>50	>50	>50	>50	>50
34	Tamoxifen	3.69	4.38	4.39	19.1	10.68

*Pet: petroleum ether, **Ch: chloroform fraction, ***Met: methanol fraction
N: Not done

against MDBK, MCF-7 and A-549 (IC_{50} 4.84-21.42 µg/mL) whereas the chloroform fraction was only toxic to MCF-7 and MDBK cells, suggesting these fractions as interesting sources for further biological investigations. *Juniperus excelsa* was traditionally used for dysmenorrhea, cough, bronchitis and common cold, jaundice and tuberculosis [15]. The crude methanol extract of *J. excelsa* has shown significant antitumor activity with inhibition of 86.6% and the di-ethyl ether fraction has demonstrated the lowest antitumor activity with inhibition of 46.6% in a previous study [16].

Also cytotoxic activity of the hexane extract of *J. excelsa* has been investigated against series of cell lines and has been found to be highly active against LNCaP, KB-V (+VLB) and KB-V (−VLB) cell lines [17].

Previous studies have revealed the *in vitro* anticancer activity of *Avicennia marina* extract on various cancer cell lines (HL-60, HepG-2, NCI-H23 and HEK-293T) determined by MTT assay. The results demonstrated that the methanol and aqueous extract of *A. marina* showed cytotoxicity against HL-60 and NCI-H23 cell line with efficient IC_{50} values but exhibited negligible toxicity against the normal cell line (HEK-293T) [18]. In another study, the methanol extract of *Periploca aphyla* demonstrated a remarkable antioxidant and cytotoxic activity due the presence of bioactive constituents [19]. Also the petroleum ether extract of *Salvadora persica* has presented IC_{50} values 43.6, 44.3, 19.87, 10.2 µg/mL against HepG-2, MCF-7, A-549 and HCT-116, respectively [20].

The cytotoxic effects of an ethanol extract of *Teucrium polium* on four cell lines has been investigated and have been shown that the ethanol extract of *T. polium* could suppress the growth of the evaluated cell lines effectively. The IC_{50} values for each cell line were calculated as 90 µg/mL for A-549, 106 µg/mL for BT-20, 140 µg/mL for MCF-7, and 120 µg/mL for PC-12 cells [21]. The *n*-hexane, chloroform, chloroform-methanol, butanol, methanol-water and aqueous extracts of *Ziziphus spina-christi* have significantly and concentration-dependently reduced the viability of Hela and MAD-MB-468 cells. In both cell lines, chloroform-methanol extract of *Z. spina-christi* has been more potent than other extracts [22].

Except for *Calotropis procera* and *Juniperus excels*, other species did not show cytotoxicity in our study which is not in accordance which previous studies. It could be concluded that the plants collected from different locations might contain various constituents resulting in different biological activities. Moreover, the plant extracts might have shown selective *in vitro* cytotoxicity against some cell lines and were not toxic to other cells. There is also another possibility that these species might have presented their cytotoxicity by a mechanism that could not be detected by MTT assay hence other methods of evaluation could be suggested for 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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