

## Cytotoxic Effects of the Extracts of Iranian *Taxus baccata* and *Cupressus horizontalis* on Cancer Cells

Hojjat Sadeghi-aliabadi\*<sup>a</sup>, Seyed Ahmad Emami<sup>b</sup>, Morteza Saeidi<sup>a</sup>, Abbas Jafarian<sup>c</sup>

<sup>a</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences and Health services, Isfahan, Iran.

<sup>b</sup>Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Mashhad University of Medical Sciences and Health services, Mashhad, Iran. <sup>c</sup>Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Science and Health services, Isfahan, Iran.

---

### Abstract

It has been reported that several conifers possess cytotoxic activities on some human tumor cell lines. Taxol as a natural cytotoxic compound has been extracted from this family. In a program to screen the cytotoxic effects of natural resources, male and female branchlets, fruit or bark of two different species of Iranian conifers were collected, identified and the cytotoxic effects of their hydroalcoholic extracts on three human tumor cell lines were determined. Different concentrations of extracts were added to cultured cells and incubated for 72 h. Cell survival was evaluated using MTT assay. Extracts from bark of female *Taxus baccata* showed inhibitory activities against Hela cells. The extracts of the branchlets of male and female *T. baccata* and branchlets of *Cupressus horizontalis* showed inhibitory activities against MDA-MB-468 cells, whereas the extracts of branchlets of female *T. baccata* showed inhibitory activities against KB cells. In conclusion, extract obtained from the bark of Iranian *T. baccata* showed a comparable cytotoxic effect to doxorubicin against Hela cells.

**Keywords:** *Taxus baccata*; *Cupressus horizontalis*; Cytotoxicity; Cancer cells.

---

### Introduction

Isolation and identification of some potent anti-tumor compounds such as colchicine, *Vinca* alkaloids and recently paclitaxel (taxol<sup>®</sup>) from medicinal plants, has encouraged scientists to screen different parts of plant species against cancer cell lines. Biological methods (due to their reliability, simplicity and sensitivity) have been extensively used for evaluation of natural products, in the last three decades. There are about 10 species of Iranian conifers, which are mostly found in the northern parts of the country. Previous preliminary studies carried out by this group on some of these species have revealed that different parts of these species possess cytotoxic effects against a number of human

tumor cell lines. The potent compound present within some of tested species was podophylotoxin, whereas in other species a previously undescribed but related lignan, silicicolin, now called "desoxypodophylotoxin" was the active component. Further investigation on the leaves of several genera of conifers (*Taxus*, *Platyclusus*, *Libocedrus*, *Podocarpus*, *Chamacyparis* and *Callitris*) confirmed the presence of cytotoxic compounds or tumor necrosing substances (1, 2). Cytotoxic effects of *Juniperus sabina* and *Platyclusus orientalis* extracts against Hela cells (3, 4), ethanolic extracts of *J. phoenicea*, *J. bermudiana*, *J. communis* and *Libocedrus decrrens* against KB cell lines (5, 8) have been previously reported. Literature reviews revealed that no study has been carried out on the cytotoxicity of extracts of Iranian *T. baccata* and *C. horizontalis* in

\* Corresponding author:

E-mail: sadeghi@pharm.mui.ac.ir

cancer cell lines. This study was conducted to evaluate the cytotoxic effects of different parts of the mentioned plants.

## Experimental

### Plant material

Terminal branchlet of male and female trees, fruits and bark of *T. baccata* and *C. horizontalis* were collected from the northern parts of Iran (Ghozlogh and Soorkesh in Golestan province and Sangdehe in Mazandaran province) in September 2000. Plants were identified by the Department of Botany, Tehran University, Iran. The plant material was stored at  $-20^{\circ}\text{C}$  before use. Voucher specimenes of the plants were deposited in the herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

### Extraction and isolation

50 g of each plant parts was crushed and soaked in 75 ml of ethanol (80% v/v) for 24 hr and then percolated (5 h, 30 drops/min)<sup>9</sup>, Extracts were concentrated by rotary evaporator and dried in an oven at  $40^{\circ}\text{C}$  to give 0.5-0.8 g of a solid residue. 0.02 g of this solid residue was then dissolved in 100 ml of water containing 0.1% of ethanol, filtered and sterilized using  $0.22\mu$  microfilters. Dilution was continued in order to obtain final extract concentrations of 10 and 20  $\mu\text{g}/\text{ml}$ .

### Cell lines

Hela (Human cervix carcinoma), KB (Human Caucasian/ epidermal carcinoma) and MDA-MB-468 (Human breast adenocarcinoma) cell lines were purchased from Pasture Institute, Tehran, Iran. They were grown in RPMI-1640 [each 500 ml of RPMI-1640 was supplemented with 10% of fetal calf serum, 5 ml of penicillin/streptomycin (50 IU  $\text{ml}^{-1}$  and 500  $\mu\text{g ml}^{-1}$  respectively), 5 ml of sodium pyruvate (1 mM),  $\text{NaHCO}_3$  (1 g) and 5 ml of L-glutamine (2 mM)]. The final medium was then sterilized using  $0.22\mu$  microfilters and stored at  $4^{\circ}\text{C}$  before use.

### Maintenance of the human cell lines

Cell line was maintained and grown in RPMI 1640 up to 15 subcultures. A sample of each cell lines was frozen and kept under liquid nitrogen for future studies.

### MTT-based cytotoxicity assay

The cytotoxic effect of extracts obtained against previously mentioned human tumor cell lines was determined by a rapid colorimetric assay, using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) and compared with the untreated controls (10). This assay is based on the metabolic reduction of soluble MTT by mitochondrial enzyme activity of viable tumor cells, into an insoluble coloured formazan product, which can be measured spectrophotometrically after dissolving in dimethylsulfoxide (DMSO) (11). Briefly, 200  $\mu\text{l}$  of cells ( $5 \times 10^4$  cells per ml of medium) were seeded in 96 microplates and incubated for 24 h ( $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$  air humidified), then 20  $\mu\text{l}$  of prepared concentrations of each extract was added. Microplates containing cells and extracts were incubated for another 72 h in the same condition. Doxorubicin was used as a positive control. The first column of each microplate was assumed as the negative control (containing no extracts or doxorubicin). To evaluate cell survival, 20  $\mu\text{l}$  of MTT solution (5 mg/ml in phosphate buffer solution) was added to each well and incubated for 3 h. 150  $\mu\text{l}$  of an old medium containing MTT was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined at 540 nm by an ELISA plate reader. Each extract concentration was assayed in 8 wells and repeated 6 times. Standard curves (absorbance against number of cells) for each cell line were plotted. Intraday and interday variations were determined. Cell survival percentage was calculated based on standard curves. Percentage of cell survival in the negative control was assumed as 100.

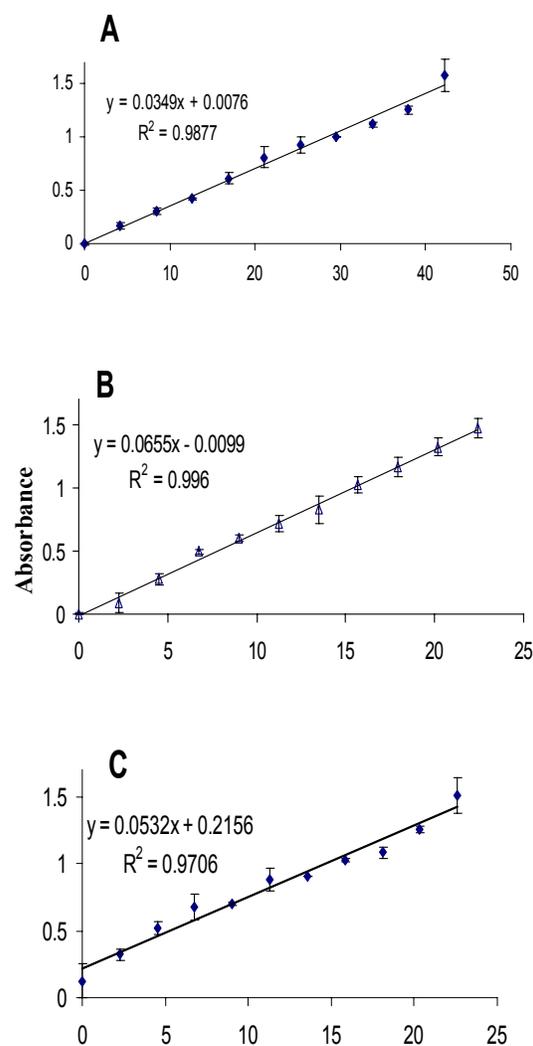
### Statistical analysis

SIGMASTAT<sup>TM</sup> (Jandel Software, San Raphael, CA) was used to perform statistical tests. Analysis of variance followed by Duncan test was used to distinguish the differences among groups. Significance was assumed at 5% level.

## Results and Discussion

For Hela, KB and MDA-MB-468 cell lines a good relationship between the number of cells and absorbance were observed ( $r^2=0.9879$ ,  $0.9967$  and  $0.9618$ , respectively) (Figure 1). Intraday and interday variations for all standard curves were acceptable (%CV < 20). Doxorubicin as a positive control showed significant inhibitory effects (cell survival less than 50%) against all the tested cell lines. Extracts obtained from the bark of female trees (10 and 20  $\mu\text{g/ml}$ ) showed inhibitory effects against Hela cells. Extracts from the branchlets of female *T. baccata* trees 10 and 20  $\mu\text{g/ml}$  showed inhibitory effects against KB cells. Extracts from the branchlets of female *T. baccata* and *C. horizontalis* trees (20  $\mu\text{g/ml}$ ) showed inhibitory effects against MDA-MB-468 cells (Table 1).

Extract obtained from the bark of female *T. baccata* tree was found to have the most significant inhibitory effect against Hela cells. This effect was comparable with doxorubicin at the same concentration. Different alkaloids such as cephalomanin, taxin B, harringtonin and bacatin has been isolated from *T.* species and their cytotoxic effects were studied (1, 12, 13). Emami and Asili (14) showed that the branchlets of female *T. baccata* tree had a greater amount of alkaloid than that of the branchlets of male tree. Also, other studies have shown that alkaloids were involved in cytotoxic activities of conifers (1, 15). This probably indicates that alkaloids are involved in the cytotoxic effects of *T. baccata*. This suggestion is confirmed by the results obtained from extracts of branchlets and fruits of *C. horizontalis* against MDA-MB-468 cells. It seems that saponins, tannins and flavonoids are not responsible for the cytotoxic effects of



**Figure 1.** Relationship between cell numbers and absorbance, using MTT assay. Absorbance was determined at 540 nm. Numbers are shown as arbitrary units (A=Hela, B=KB, C=MDA-MB-468, (n=6).

plants tested, as the amount of these compounds are not consistent with the cytotoxic effects of extracts of different plant parts (14).

**Table 1.** Cytotoxic effects of hydroalcoholic extracts of different parts of Iranian conifers against 3 cancer cell lines following 96h continuous exposure to each extract.

Cell lines	Plants						Controls	
	<i>T. baccata</i>				<i>Cupressus horizontalis</i>		Positive (Doxorobin)	Negative
	Branchlets (female)	Branchlets (male)	Bark (female)	Fruits	Branchlets	Fruits		
Hela	80.1 ± 5.2 <sup>a*</sup>	75.1 ± 5.5 <sup>a*</sup>	35.7 ± 1.7 <sup>a*</sup>	83.3 ± 3.0 <sup>a*</sup>	74.1 ± 6.3 <sup>a*</sup>	75.4 ± 5.0 <sup>a*</sup>	24.7 ± 1.1 <sup>a*</sup>	100 ± 5.4
	61.8 ± 7.8 <sup>ba*</sup>	50.1 ± 3.1 <sup>a*</sup>	26.6 ± 3.0 <sup>a*</sup>	70.8 ± 10.8 <sup>a*</sup>	58.8 ± 5.2 <sup>a*</sup>	71.3 ± 6.8 <sup>a*</sup>		
KB	41.6 ± 2.9 <sup>a*</sup>	107.1 ± 2.1	82.2 ± 9.6 <sup>a*</sup>	107.9 ± 4.0	93.4 ± 9.0	104.3 ± 8.0	14.1 ± 1.5 <sup>a*</sup>	100 ± 4.6
	35.2 ± 5.0 <sup>a*</sup>	74.4 ± 2.1 <sup>a*</sup>	68.2 ± 9.9 <sup>a*</sup>	102.5 ± 9.6	90.1 ± 3.6 <sup>a*</sup>	95.7 ± 6.9		
MDA-MB-468	53.3 ± 10.5 <sup>a*</sup>	83.1 ± 5.5 <sup>a*</sup>	77.2 ± 16.5 <sup>a*</sup>	90.7 ± 3.7 <sup>a*</sup>	54.5 ± 5.1 <sup>a*</sup>	67.8 ± 7.0 <sup>a*</sup>	3.8 ± 0.7 <sup>a*</sup>	100 ± 3.1
	40.9 ± 2.8 <sup>a*</sup>	50.8 ± 5.6 <sup>a*</sup>	70.3 ± 13.1 <sup>a*</sup>	85.1 ± 8.6 <sup>a*</sup>	36.7 ± 3.2 <sup>a*</sup>	57.2 ± 5.0 <sup>a*</sup>		

Values are mean ± SD (n = 6)

\* = p < 0.05

a: Extract concentration at 10  $\mu\text{g/ml}$

b: Extract concentration at 20  $\mu\text{g/ml}$

### Acknowledgment

This study was supported by a grant from the research council of Isfahan University of Medical Sciences, Isfahan, Iran. Authors are thankful to the late Dr. K. Javanshir, University of Tehran for identification of the plant material.

### References

- (1) Oliver Huang CH, Kingston DI, Magri NF and Samaranyake G. New taxanes from *Taxus brivifolia*. *J. Nat. Prod.* (1986) 49: 665-669
- (2) Uden WV, Homan B, Woerdenbag HJ, Pras N and Malingre TM. Isolation, purification and cytotoxicity of 5-methoxypodophylotoxin. *J. Nat. Prod.* (1992) 55: 102-110
- (3) San Feliciano A, Miguel Del Corral JM, Gordaliza M and Castro A. Lignans from *Juniperus sabina*. *Phytochemistry* (1990) 29: 1135-1138
- (4) Kosuge T, Yokota M, Sugiyama K, Saito M, Iwata Y, Nakura M and Yamamoto T. Study on anticancer principle in Chinese medicines. *Chem. Pharmac. Bull.* (1985) 33: 5565-5567
- (5) Carines DA, Ekundayo O and Kingston GI. Lignans from *Juniperus phoenicea*. *J. Nat. Prod.* (1980) 43: 495-497
- (6) Cole JR, Tammami B and Torrance J. Antitumor agent from *Juniperus bermudiana*. *Phytochemistry* (1977) 16: 1100-1110
- (7) Markkanen T. Antihyperthermic agent from *Juniperus communis*: its purification, identification and testing in primary human amnion cell culture. *Drugs Exp. Clin. Res.* (1981) 7: 691-697
- (8) Morris KS, Hemingway RJ and Hemingway JC. Tumor inhibitors XIX: Desoxypodophylotoxin, the cytotoxic principle of *Libocedrus decurrens*. *J. Nat. Cancer Inst.* (1967) 56: 408-409
- (9) List H and Schmidt P. *Technologie Pflanzlicher Arzneizubereitungen*, Wissenschaftliche Verlagsgesellschaft mbh, Stuttgart (1984) S.140
- (10) Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Meth.* (1983) 65: 55-63
- (11) Carmichale J, De Graff WG, Gazdar AF, Mina JD and Mitchell JB. Evaluation of a tetrazolium based semiautomated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Res.* (1987) 47: 936-942
- (12) Hoffman EJ. *Cancer and the Search for Selective Biochemical Inhibitors*. CRC Press, Washington DC (1999) 2-18
- (13) Ma W, Park GL, Gomez GA and Nieder MH. New bioactive taxoids from cell cultures of *Taxus baccata*. *J. Nat. Prod.* (1994) 57: 116-122
- (14) Emami SA and Asili J. *Phytochemical Evaluation and Antimicrobial Effects of Essential Oils of Iranian Conifers*, (Pharm. D. thesis), School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran (1997)
- (15) Mc Lauphlin JL, Miller RW, Powell RG and Smith CR. 19-Hydroxy baccatin III, 10-deacetylcephalomannine and 10-deacetylaxol: new antitumor taxanes from *Taxus wallichiana*. *J. Nat. Prod.* (1981) 44: 312-31