



## Cytotoxic Effects of Five Species of *Inula* Against Some Tumor Cell Lines

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### Abstract

The chloroform soluble fractions of ethanolic extracts of five *Inula* belonging to the Compositae family were evaluated for cytotoxic activity against five different cell lines including CACO2 (human colon adenocarcinoma), MCF7 (human breast adenocarcinoma), HEPG2 (human hepatocellular carcinoma), VERO (green African monkey kidney) and WEHI164 (balb c mouse fibrosarcoma). Cytotoxicity was assessed by MTT assay. Among these five species, *Inula oculus christi*, exhibited better cytotoxic effects.

**Keywords:** Compositae; Cytotoxicity; *Inula*; *Inula oculus christi*; MTT assay.

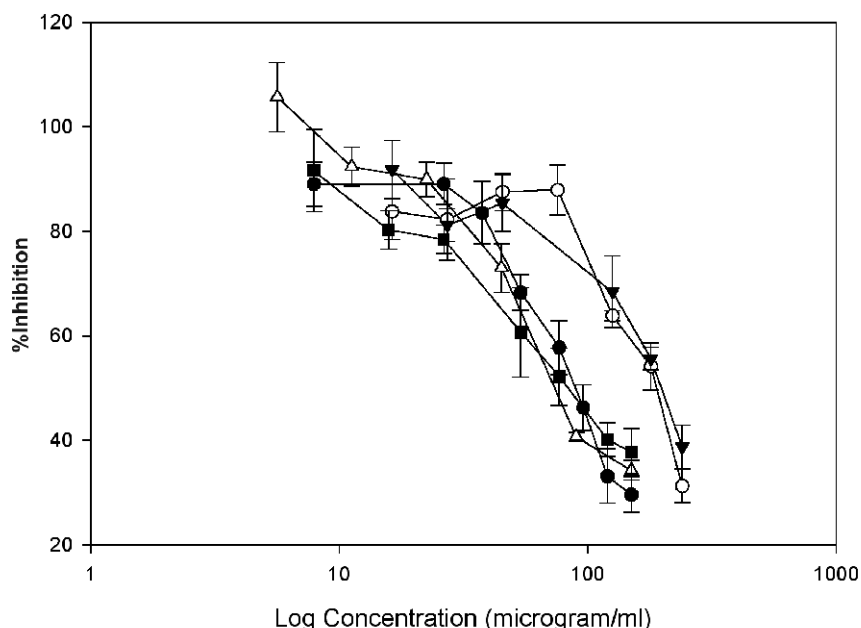
**Received:** June 18, 2006; **Accepted:** August 22, 2006.

### 1. Introduction

*Inula* is a genus with 14 species growing in Iran. These plants have been used for bronchial complaints with profuse phlegm, nausea and vomiting, hiccups, and flatulence [1]. *Inula helenium* L. (Compositae) and *I. racemosa* Hook f. have been used in traditional Chinese medicine for abdominal distension and pain, acute enteritis and bacillary dysentery [2]. Alantolactone and isoalantolactone derived from these plants have shown significant anti-inflammatory and hepatoprotective activities similar to silymarin [3]. *I. racemosa* was used in folk medicine of East Asia and Europe to cure myocardial ischemia [4]. *I.*

*confertiflora* has been used in Africa for treatment of skin diseases for a long time [5]. Furthermore, cytotoxicity studies have been carried out in different species of *Inula*, and some of these have led to the isolation of good cytotoxic agents, such as 1-O-acetylbritannilactone, 1,6-O,O-diacetylbritannilactone and ergolide in *I. Britannica* L. [6-8], 1,6- $\alpha$ -dihydroxy-4- $\alpha$ -H-1,10-secoeudesma-5(10),11(13)-dien-12,8-beta-olide from *I. japonica* Thunb. [9], eudesmanolides from *I. graveolens* L. [10], germacranolides from *Inula verbascifolia* subsp. *methanea* (Hauskn) Tutin [11], sesquiterpene lactones from *I. helianthus aquatica* C.Y Wu ex Ling., Compositae, *I. helenium* L. [12] and *I. hupehensis* Ling. [13]. Studies on this genus is still going on and in the present research the cytotoxic effects of five species of *Inula* including: *I. granitoides*

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**Figure 1.** Dose-response curves of different extracts in MCF7 cells (human breast adenocarcinoma). The cells were treated with the extracts at indicated concentrations for 24 h and cell viability was determined using the MTT colorimetric assay, as described in Materials and methods. Values represent the mean  $\pm$  SE of three separate experiments. Symbols: ( $\Delta$ ) *I. oculus christi*; ( $\square$ ) *I. vulgare*; ( $\circ$ ) *I. thapsoides*; ( $\blacktriangledown$ ) *I. salicina*; and ( $\bullet$ ) *I. granitoides*.

Boiss., *I. vulgare* LAM., *I. thapsoides* M.B.ex WILLD., *I. oculus christi* L., and *I. salicina* L. subsp. *aspera* POIR., have been evaluated against some tumor cell lines.

## 2. Materials and methods

### 2.1. Plant material

Five species belonging to Compositae family were collected from different parts of Iran as follows: *Inula granitoides* in 2005 from Qeshm (southern Iran), *I. vulgare* in 2005 from Golestan province (northern Iran), *I. thapsoides* in 2004 from Golestan province, *I. oculus christi* in 2005 from North Khorasan province and *I. salicina*, in 2005 from West Azerbaijan province (north-west of Iran).

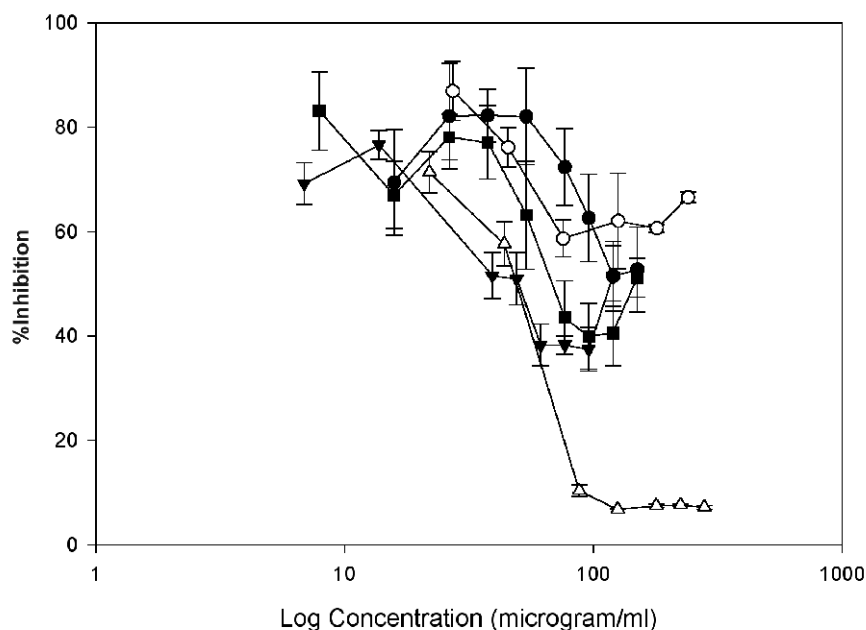
The aerial parts of these plants were dried and soaked in 96% EtOH at the room temperature for three days. Each day the solvent was replaced with fresh solvent. The chloroform-soluble fraction of each ethanolic extract was then used in the cytotoxicity assays.

### 2.2. Cell lines

Different tumor cell lines including: CACO2 (human colon adenocarcinoma), MCF7 (human breast adenocarcinoma), HEPG2 (human hepatocellular carcinoma), VERO (green African monkey kidney) and WEHI164 (balb c mouse fibrosarcoma) were obtained from Pasture Institute, Tehran, Iran. Each cell line was cultured in suitable medium to maintain the desired growth, plus 10% FBS (20% for cell line: CACO2) and 1% penicilline-streptomycine, in a humidified incubator at 37 °C in an atmosphere of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The growth curve of each cell line was accessed.

### 2.3. MTT assay

Cell viability was assessed in a microculture tetrazolium/formazan assay (MTT assay), according to the method of Alley *et al.* [14] with some modifications, in the absence and presence of different concentrations of the plants extracts. The plants extracts were prepared in 0.4% DMSO. The cells were seeded in 96-well plates at



**Fig 2.** Dose-response curves of different extracts in HEPG2 cells (human hepatocellular carcinoma). The cells were treated with the extracts at indicated concentrations for 24 hours and cell viability was determined using the MTT colorimetric assay, as described in Materials and methods. Values represent the mean  $\pm$  SE of three separate experiments. *Inula oculus christi* exhibited better results comparing to other plant extracts. Symbols: ( $\Delta$ ) *I. oculus christi*; ( $\square$ ) *I. vulgare*; ( $\circ$ ) *I. thapsoides*; ( $\blacktriangledown$ ) *I. salicina*; and ( $\square$ ) *I. granitoides*.

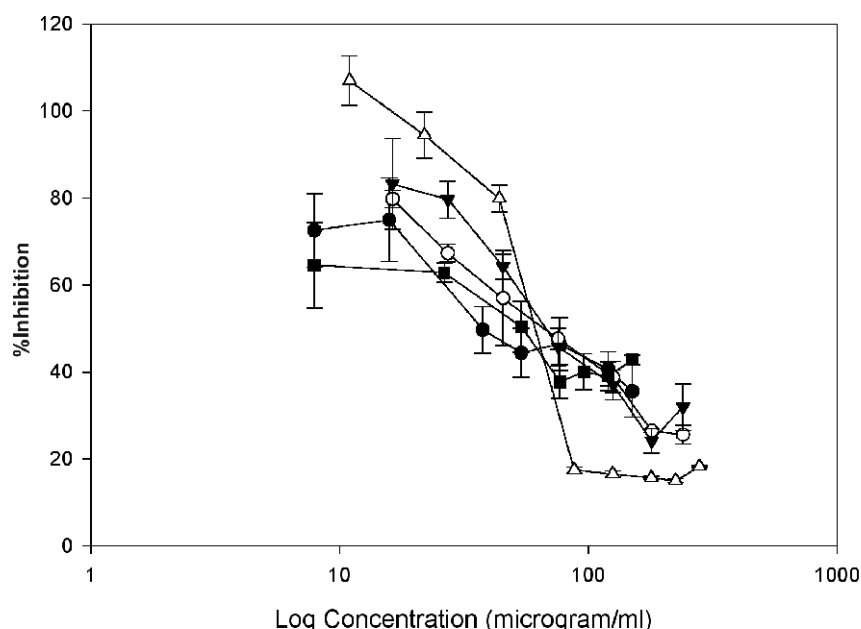
$6 \times 10^3$  for VERO cells,  $10 \times 10^3$  for CACO2 and HEPG2 cells and  $15 \times 10^3$  for MCF7 and WEHI164 cells. Four wells for each concentration were seeded and triplicate plates were used for each cell line. The cells were then incubated at 37 °C. After 24 h the medium was replaced with fresh medium containing different concentrations of the plants extracts (5-280  $\mu\text{g/ml}$ ). After 24 h exposure of the cells to the plant extracts at 37 °C, the medium was replaced with fresh medium containing MTT ([3-(4,5-dimethyl thiazol-2-yl)-2,4- diphenyl tetrazolinm bromide]) from Sigma Chemical Company,

with a final concentration of 0.5 mg/ml, and the cells were incubated for another 4 h in a humidified atmosphere at 37 °C, then the medium containing MTT was removed and the remaining MTT-formazan crystals were dissolved in DMSO. The absorbance was recorded at 570 nm immediately using an ELISA reader. The  $\text{IC}_{50}$  was then defined as the concentration of the extract that produced a 50% decrease in cell viability relative to the control which was wells exposed to the solvent without any extract.

**Table 1.** *In vitro* cytotoxicity of plant extracts.

Cell line Plant	$\text{IC}_{50}$ ( $\mu\text{g/ml}$ )				
	VERO	WEHI	CACO2	MCF7	HEPG2
<i>Inula oculus christi</i>	17.96 $\pm$ 1.09	49.31 $\pm$ 1.11	66.06 $\pm$ 1.08	67.37 $\pm$ 1.15	31.27 $\pm$ 1.1
<i>I. thapsoides</i>	N.G.	470.6 $\pm$ 1.48	62.37 $\pm$ 1.04	221.51 $\pm$ 1.24	N.G.
<i>I. salicina</i>	N.G.	N.G.	74.30 $\pm$ 1.08	285.94 $\pm$ 1.43	41.51 $\pm$ 1.11
<i>I. vulgare</i>	306.71 $\pm$ 1.28	N.G.	79.83 $\pm$ 1.34	103.78 $\pm$ 1.16	N.G.
<i>I. granitoides</i>	860.04 $\pm$ 1.65	N.G.	70.80 $\pm$ 1.19	79.15 $\pm$ 1.13	92.41 $\pm$ 1.20

\*N.G.: not good dose response correlation was observed.



**Figure 3.** Dose-response curves of different extracts in CACO2 cells (human colon adenocarcinoma). The cells were treated with the extracts at indicated concentrations for 24 h and cell viability was determined using the MTT colorimetric assay. Values represent the mean±SE of three separate experiments. *Inula oculus christi* exhibited a better cytotoxic effect. Symbols: (Δ) *I. oculus christi*; (◐) *I. vulgare*; (○) *I. thapsoides*; (▼) *I. salicina*; and (◑) *I. granitoides*.

### 3. Results and discussion

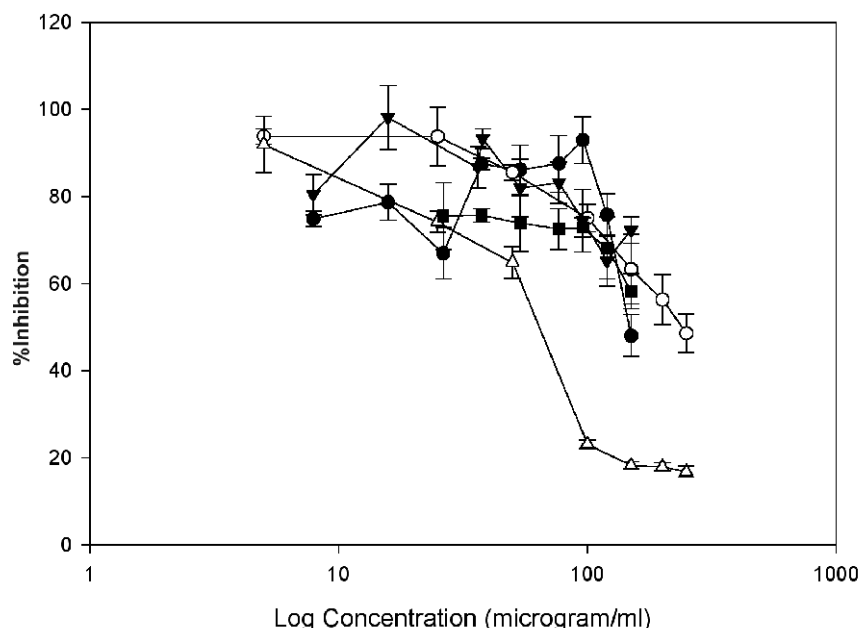
The effects of the chloroform-soluble fraction of the extracts on different cell lines are shown in Table 1. According to the IC<sub>50</sub>s, the extract of *Inula oculus christi* with the IC<sub>50</sub>s of 17.96±.09, 31.27±.1, 49.31±.11, 66.06±.08, 67.37±.15, μg/ml for VERO, HEPG2, WEHI164, CACO2, MCF7 cell lines, respectively, exhibited higher cytotoxic effects than extracts from other plants studied in this investigation.

The effects of different extracts on each cell line, is demonstrated in Figures 1 to 5. In Figure 1, the effects of extracts on MCF7 cells are demonstrated. Here *I. oculus christi*, *I. granitoides* and *I. vulgare*, demonstrated better cytotoxic effects compared to the other two extracts. Among these three, *I. oculus christi* showed lower IC<sub>50</sub>. In Figure 2, the effects of different extracts on HEPG2 cells were examined and *I. oculus christi* exhibited better cytotoxic effects. As shown in Figure 3, the responses of CACO2 cell line are similar to different extracts to some extent, but

*I. oculus christi* exhibits a better response than other extracts. Comparing the cytotoxic effects of the extracts on WEHI 164, three of the extracts (*I. vulgare*, *I. granitoides*, *I. salicina*) did not show good cytotoxic effects. Again *I. oculus christi* exhibited better results comparing to other extracts (Figure 4). As it is shown in Figure 5, *I. oculus christi* exhibited cytotoxic effects in much lower concentrations comparing to other plants in VERO cells.

In this study, we were looking for the most cytotoxic plant among the five species of *I.* which were collected from different parts of Iran. The plant extracts were treated against five cell lines for determination of their cytotoxic activity and the IC<sub>50</sub>s were assessed. The IC<sub>50</sub>s for each cell line were compared using ANOVA statistical analysis, and they were showed to be significantly different in each cell line with  $p < 0.001$ .

*I. oculus christi* seemed to be more potent regarding the IC<sub>50</sub>s in cell lines: VERO, WEHI164, MCF7, HEPG2 and was only less



**Figure 4.** Dose-response curves of different extracts in WEHI164 cells (balb c mouse fibro sarcoma). The cells were treated with the extracts at indicated concentrations for 24 h and cell viability was determined using the MTT colorimetric assay. Values represent the mean  $\pm$  SE of three separate experiments. *Inula oculus christi* showed a better dose response curve and also better results comparing to other extracts. Symbols: ( $\Delta$ ) *I. oculus christi*; ( $\square$ ) *I. vulgare*; ( $\circ$ ) *I. thapsoides*; ( $\blacktriangledown$ ) *I. salicina*; and ( $\bullet$ ) *I. granitoides*.

potent in cell line: CACO2 comparing to *I. thapsoides*, but it still showed more potency than other extracts considering this specific cell line.

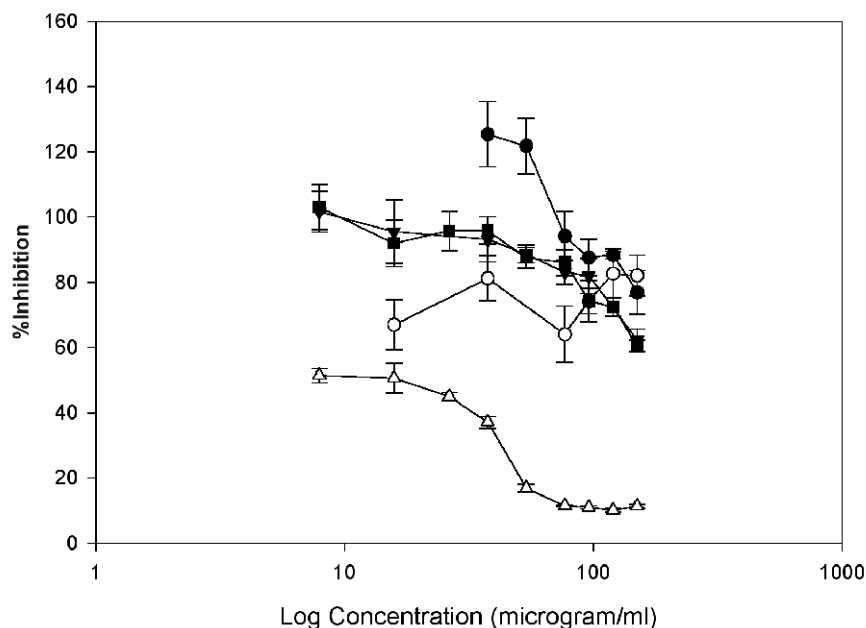
Taking these results into consideration, *I. oculus christi* was determined worth to continue studies and was selected for further bioassay guided fractionation.

### Acknowledgments

The authors wish to thank Mr. A.Ghorbani, Traditional Medicine and Material Medica Research Center, Shaheed Beheshti University of Medical Sciences, for his assistance in collecting, identifying and scientific nomenclature of the plants.

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**Figure 5.** Dose-response curves of different extracts in VERO cells (green African monkey kidney). The cells were treated with the extracts at indicated concentrations for 24 h and cell viability was determined using the MTT colorimetric assay. Values represent the mean  $\pm$  SE of three separate experiments. *Inula oculus christi* exhibited lower  $IC_{50}$  in comparison with other plant extracts. Symbols: ( $\Delta$ ) *I. oculus christi*; ( $\circ$ ) *I. vulgare*; ( $\square$ ) *I. thapsoides*; ( $\blacktriangledown$ ) *I. salicina*; and ( $\blacksquare$ ) *I. granitoides*.

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