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*Full Length Research Paper*

# Antitumor and antibacterial activity of four fractions from *Heracleum persicum* Desf. and *Cinnamomum zeylanicum* Blume

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**At the present study tumor inhibition and antibacterial activity of the essential oils of *Heracleum persicum* and *Cinnamomum zeylanicum* was investigated. Methanol and petroleum ether were extracted from *C. zeylanicum* by potato disk method. These fractions showed cytotoxic effects in brine shrimp lethality assay (BSL). The authors found both *H. persicum* (57.16%) and *C. zeylanicum* (72.90%) had inhibition effects on *Agrobacterium tumefaciens* which induced crown gall tumor on potato disk. These oils also exhibited antitumor activity where $IC_{50}$ was applied and the values were 2.24 and 1.20 mg/mL, respectively, for *H. persicum* and *C. zeylanicum*. *C. zeylanicum* also inhibited the growth of all tested Gram- positive and Gram-negative strains. In all, the findings of the present study completely correspond to the results obtained in brine shrimp lethality.**

**Key words:** Crown gall, *Agrobacterium tumefaciens*, *Heracleum persicum*, *Cinnamomum zeylanicum*, essential oil.

## INTRODUCTION

Medicinal plants especially those have been used as condiment are a part of daily foods in several places in the world. The toxicity of common used spices, whether as a condiment or for therapeutic uses, is an important consideration. The safety of the spices has been considered scarcely. Furthermore, the plants with anticancer activity have been interested in phytotherapy for many years and many of natural compounds especially from medicinal plants have been considered as leader for new antitumor drugs. There are many accepted advanced screening by national cancer alliance (NCA) methods. They can be used to minimize the cost and time in order to access any novel anticancer agents in screening process. The crown gall tumor assay (CGTA) was one of these bioassays methods.

The inhibition of *Agrobacterium tumefaciens*-induced tumors (Crown Gall) in potato disc tissue is an assay based on anti mitotic activity and it is used for searching the new antitumor agents. Crown gall is a neoplastic plant disease caused by *A. tumefaciens* (Ferrigni et al., 1982; McLaughlin et al., 1993; Das et al., 2007). The present work aims to evaluate antitumor and antibacterial activity of four fractions from medicinal plants of *Heracleum persicum* and *Cinnamomum zeylanicum*

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**Abbreviations: CGTA,** Crown gall tumor assay; **MIC,** minimum inhibitory concentration.

which have been reported to be cytotoxic in BSL assay (Sharififar et al., 2009a).

*H. persicum* Desf. Ex and Fischer belongs to *Apiaceae* family, known as "golpar". It is native to Iran and used as carminative. Fruits of the plant are used as a condiment and as a constituent of the daily diet of general population in Iran. Its fruits are consumed widely as antiflatulence and antimicrobial (Aynehchi, 1991. *H. persicum* contains volatile oils, flavonoids and furanucoumarins (Ghodsi, 1976; Merijanian et al., 1980). Anticonvulsant and cytotoxicity of this plant has been reported (Moshafi et al., 2010; Sayyah et al., 2005). Anticonvulsant and immune-stimulant activities are also reported for the plant (Sayyah et al., 2005; Sharififar et al., 2009b). Cinnamon *(C. zeylanicum Blume,* Loraceae) has widely been used in traditional medicine and in Iranian diet. It also exhibits a variety of biological activities including antioxidant activity, antimicrobial activity, Anti-diabetic and ovicidal activities (Jayaprakasha et al., 2006; Matan et al., 2006; Kim et al., 2006; Yang et al., 2005). The major component of the essential oil of this plant is cinnamic aldehyde which its cytotoxicity has been reported (Kwon et al., 1998).

## MATERIALS AND METHODS

### Extraction and essential oil isolation

*H. persicum* was collected from Lalehzar region in Kerman province, Iran (June, 2008) and cinnamon was purchased from a local market. The plants were authenticated in department of Pharmacognosy, Faculty of Pharmacy, Kerman, Iran. A voucher specimen of *H. persicum* (KP1185) was deposited in the herbarium center in department of Pharmacognosy. First, 500 g of dried barks of cinnamon was grounded in a grinder with a 2 mm diameter mesh. Then, it was extracted with petroleum ether, chloroform, diethyl ether, methanol and water using percolation method for 72 h for each solvent. Solvent removal carried out under vacuum until drying. Methanol and petroleum ether extracts were used for antitumor assay. Barks of cinnamon and fruits of golpar (100 g) were submitted for 4 h to water-distillation using a Cleavenger apparatus separately. The obtained essential oils were dried over anhydrous sodium sulphate, then stored at 4°C until tested and analyzed. The essential oils of these plants were used for antitumor tests.

### Antibacterial activity

The antibacterial activity of the separated fractions was studied against Gram-positive (*Staphyllococcus aureus, Bacillus subtilis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) pathogenic bacteria using broth dilution and agar dilution method for the extracts and essential oils; respectively. Antibacterial activity was expressed with the zone diameter of growth inhibition and minimum inhibitory concentration (MIC) for extracts and essential oils, respectively. First, the stock media was prepared from tested microorganism in nutrient agar, incubated in 37°C for 24 h. The assay was carried out in the agar dilution tubes. Stock solutions of the compounds in DMSO-water (4:6) were diluted to give serial two fold dilutions. 1 mL of each sample was poured into Petri plates with 9 mL sterile Muller Hinton Agar media (about 55°C), the mixture was then homogenized immediately. After cooling, 10 µL suspensions of microorganisms with concentrations adjusted to $1.5 \times 10^8$ cfu/mL were then spread onto the surface of agar containing samples. The plates spread with bacteria were incubated at 37°C for 24 h. Growth of microorganism was visually monitored. The MIC was the concentration at which no growth of microorganism was observed (Mattew et al., 2006).

### Antitumor activity

The antitumor activity of *H. persicum*, *C. zeylanicum*, petroleum ether and Methanol extracts were evaluated by potato disc bioassay method described by McLaughlin (1993). Potato (*Solanum tuberosum*) discs (1.5 x 1.0 cm), stored in ethanol 70% for two minutes and their surface sterilized by 50% sodium hypochloride for 30 min then washed with distilled water in laminar flow cabinet. The discs were inoculated with *A. tumefaciens* by immersing the discs in 48 h old broth culture of the bacterium in 100 ml medium containing 0.5 g sucrose, 0.8 g nutrient broth (DIFCO, USA) and 0.1 g yeast extract (DIFCO, USA) in water q.s. *A. tumefaciens* containing potato discs were placed into a Petri dish containing 1.5% agar.

The samples were separately dissolved in solvent. Vincristine (Albuorz Daru) (1 mg/ml) was used as the standard drug. The Petri dishes were sealed with paraffin film and incubated at 25°C for 20 h for the growth of *A. tumefaciens* and the development of crown-gall tumors on potato discs. Each set of experiment was carried out in triplicate. The crown-gall tumors developed on potato discs were stained with Lugol's solution (5% iodine plus 10% potassium iodide in water). The tumor cells lack starch, so these cells had no stains against the stained background of normal potato cells rich in starch. The crown-gall tumors on the potato discs were observed with an Olympus Microscope equipped with Olympus camera and the average number of tumors for the sample and negative control were counted on the individual discs (Haque et al., 2002).

## RESULTS AND DISCUSSION

Regarding the safety and effectiveness of natural therapies, the researchers are more interested in the use of natural medicines. In previous study, they have reported the cytotoxicity of different fractions of *H. persicum* and *C. zeylanicum* in brine shrimp lethality as the method described by Das et al. (2007), so in the present work, the antitumor and antibacterial effects of these fractions were evaluated.

### The results of antibacterial activity

*In vitro* antibacterial activity of the different fractions of *H. persicum* and *C. zeylanicum* was studied against Gram-positive (*S. aureus, B. subtilis*) and Gram-negative (*E. coli, P. aeruginosa*) pathogenic bacteria using broth dilution and agar dilution method for the extracts and essential oils, respectively. The obtained results showed that the essential oil of *C. zeylanicum* was able to inhibit

**Table 1.** Anti-bacterial activities of *H. persicum* and *C. zeylanicum* fractions observed against *A. tumefaciens* and standard strains. Minimum inhibition concentration (MIC)[a] of each fraction has been shown (mg/mL).

| Tested fraction | *A. tumefaciens* | *E. coli* | *S. aureus* | *B. subtilis* | *P. aeruginosa* |
|---|---|---|---|---|---|
| *H. persicum* essential oil (HPO) | 12.5 ± 0.2 | 25 ± 0.3 | 12.5 ± 0.1 | 6.25 ± 0.1 | 12.5 ± 0.4 |
| *C. zeylanicum* essential oil (CZO) | 12.5 ± 0.3 | 0.1* ± 0.02 | 0.2* ± 0.03 | 0.4* ± 0.01 | 0.2* ± 0.01 |
| *C. zeylanicum* methanol ex (CZM) | 20 ± 0.4 | 50 ± 1.3 | 25 ± 0.7 | >50 | 50 ± 2.9 |
| *C. zeylanicum* petroleum ex. (CZP) | 20 ± 0.7 | 50 ± 2.5 | 25 ± 1.1 | >50 | 50 ± 3.1 |

[a] Values are expressed as mean ± S.D, n = 6.

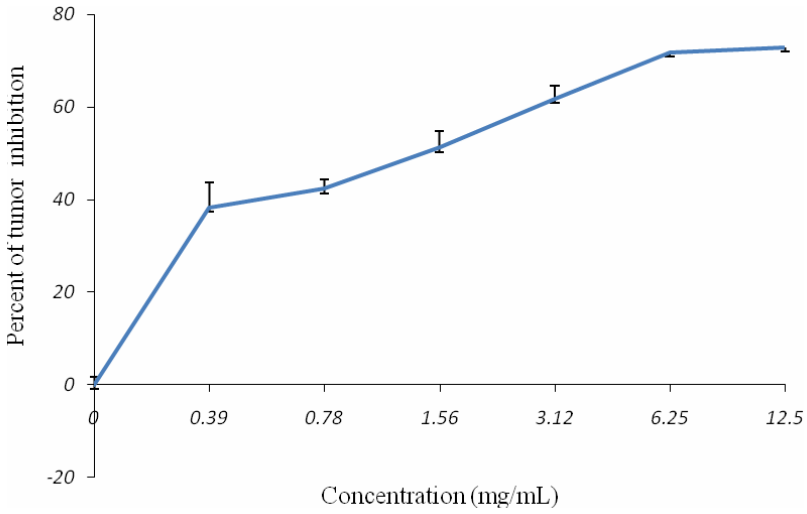


**Figure 1.** The inhibition effect of the essential oil from *C. zeylanicum* against tumor formation in potato disk.

all of the tested strains (Table 1). This activity significantly differs from the other tested samples. The most inhibition was observed against *E. coli* with MIC = 0.1 mg/ml. The inhibition against the other bacteria was determined to be in the order of: *E. coli* > *P. aeruginosa* = *S. aureus* >*B. subtilis.* The antibacterial activity of *H. persicum* was exhibited against *B. subtilis* (MIC = 6.25 mg/mL). The other fractions were inactive against tested strains and showed no significant difference ($p > 0.05$).

## The results of antitumor activity

The results of the present study showed that all the tested fractions inhibited tumor formation in the potato disc. None of the samples was able to inhibit the growth of *A. tumefaciens* otherwise high concentrations were applied (16 and 20 mg/ml for the extracts and 6.25 and 12.5 mg/ml for the essential oils). The tested fractions did not affect the viability of the *A. tumefaciens* growth. The results of the inhibition tumor and $IC_{50}$ value of the fractions are shown in Table 2. The *C. zeylanicum* and *H. persicum* fractions showed the most inhibitory tumors effects with $IC_{50}$ = 1.20 and 2.24 mg/ml, respectively. The tumors inhibitory effect for *C. zeylanicum* was 72.9% where 12 mg/mL dilution was applied (Figure 1). As shown in the figure the essential oil of the plant was able to inhibit tumor formation more than 60% in the concentration of 4 mg/mL and 70% in the concentration of 8 mg/mL. The potency of the essential oil of *H. persicum* was somehow similar to *C. zeylanicum* essential oil. HPO inhabited tumor formation 57.16% where 12 mg/ml dilution was applied (Figure 2). Although, the potency of this oil has been shown to be lesser than the former oil but even in low concentrations, noticeable tumor inhibition was considered (more than 50% inhibition in concentration of 4 mg/mL) (Figure 2). The most tumor inhibitory effect was 65.80 and 61.78% for methanol and petroleum ether extracts, respectively, where 20 mg/ml dilution applied. The obvious tumor reduction activity was observed with the CZO (72.90%) and HPO (57.16%). The activity of examined fractions

**Table 2.** Antitumor activity of tested fractions observed on potato disk.

| Test fraction | Concentration (mg/mL) | Average number of tumor/ disk | Average of tumor inhibition (%) | $IC_{50}$ (mg/mL) |
|---|---|---|---|---|
| *H. persicum* essential oil (HPO) | 12.5 | 23.88 ± 0.8[a] | 57.16 ± 1.7[a] | 2.24 |
| | 6.25 | 30.00 ± 3.2 | 56.62 ± 3.5 | |
| | 3.12 | 35.5 ± 2.2 | 50.76 ± 2.7 | |
| | 1.56 | 42.25 ± 1.7 | 37.76 ± 4.2 | |
| | 0.78 | 48.06 ± 3.6 | 30.19 ± 3.7 | |
| | 0.39 | 23.31± 1.9 | 18.17 ± 2.3 | |
| *C. zeylanicum* essential oil (CZO) | 12.5 | 17.00 ± 1.4 | 72.90 ± 3.5 | 1.20 |
| | 6.25 | 22.25 ± 1.3 | 71.80 ± 2.6 | |
| | 3.12 | 30.56 ± 1.5 | 61.89 ± 1.9 | |
| | 1.56 | 33.50 ± 2.1 | 51.34 ± 5.18 | |
| | 0.78 | 35.62 ± 3.5 | 42.37 ± 6.4 | |
| | 0.39 | 17.93 ± 1.3 | 38.42 ± 3.3 | |
| *C. zeylanicum* methanol ex. (CZM) | 20 | 22.62 ± 3.1 | 65.80 ± 3.3 | 7.80 |
| | 16 | 23.76 ± 1.2 | 61.28 ± 1.9 | |
| | 12 | 31.81 ± 0.7 | 59.86 ± 3.1 | |
| | 6 | 37.37 ± 2.0 | 44.14 ± 3.2 | |
| | 4 | 42.31 ± 1.3 | 36.88 ± 5.5 | |
| | 2 | 20.81 ± 2.3 | 29.24 ± 4.3 | |
| *C. zeylanicum* petroleum ether ex. (CZP) | 20 | 22.18 ± 1.6 | 61.75 ± 3.5 | 10.44 |
| | 16 | 24.81 ± 2.5 | 60.75 ± 4.1 | |
| | 12 | 30.31 ± 1.8 | 51.62 ± 5.7 | |
| | 6 | 33.06 ± 1.3 | 39.12 ± 3.7 | |
| | 4 | 34.68 ± 0.6 | 31.81 ± 3.7 | |
| | 2 | 17.68 ± 1.6 | 22.77 ± 4.0 | |
| Control | | 58.5 ± 1.3 | - | - |

[a] Values are expressed as mean ± S.D, n = 6.

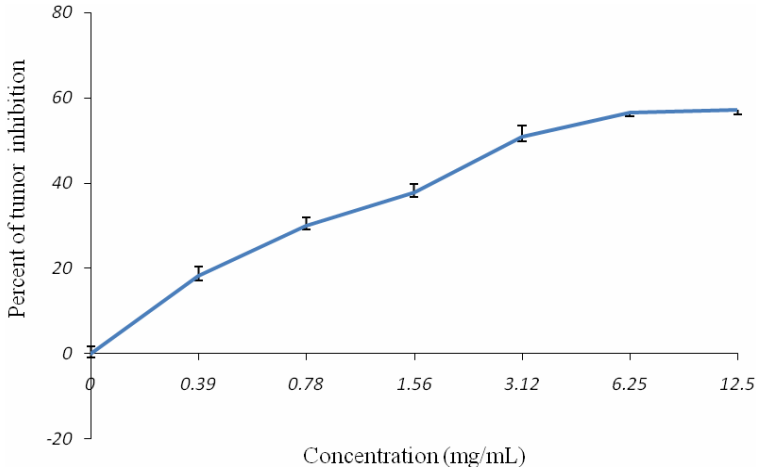


**Figure 2.** The inhibition effect of the essential oil from *H. persicum* against tumor formation in potato disk.

was based on the tumor inhibitory formation rather than the viability of the microorganism. The fractions of CZO and HPO (showed the most tumor inhibitory activity followed by methanol and petroleum ether, respectively. The cytotoxicity observed in the essential oil of *C. zeylanicum* might be because of the presences of “trans” cinnamic aldehyde in this oil (91.8%).

The cytotoxicity of transcinnamic acid has been previously reported (Kwon et al., 1998). The tumor inhibition activity of *H. persicum* could be attributed to hexyl acetate and octyl butyrate which have been reported to be the major components of the essential oil (Das et al., 2007). The cytotoxicity of the *Heracleum sphondylium* has been previously reported in the literature. The major compounds of this oil have been reported to be similar to the *H. persicum* (Ugur, 1998). The outcome of the present experiment therefore indicates that the fractions from these two plants could be regarded as a source for the development of anti tumor agents. However, further studies on more specific tumor cell lines will be necessary to confirm this hypothesis.

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