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*Full Length Research Paper*

# Antibacterial and cytotoxic activity of *Eremurus persicus* (Jaub and Spach) Boiss

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**Traditional medicine has a key role in health care worldwide. Obtaining scientific information about the efficacy and safety of the plants is one of the researcher's goals. In this research, the flowering aerial parts of *Eremurus persicus* were collected from Golpayegan (Isfahan, Iran) in May 2010. The extract was tested for its antibacterial activity against 4 Gram- positive bacteria strains (*Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus cereus, Streptococcus pyogenes*) and 5 Gram-negative bacterial strains (*Escherichia coli*, *Salmonella typhi, Shigella dysantriae, Klebsiella pneumoniae, Pseudomonas aeruginosa*). Its cytotoxic activity was also investigated using MTT assay. Here we reported the antibacterial activity of *E. persicus* against *S. aureus* (MIC = 125 mg/ml), *B. cereus* (MIC = 15.62 mg/ml), *E. coli* (MIC = 125 mg/ml), *S. typhi* (MIC = 31.25 mg/ml), *S. dysantriae* (MIC = 0.48 mg/ml) for the first time. Furthermore the cytotoxic potentials of *E. persicus* on two cell lines, HeLa and Caco-2, were studied.**

**Key words:** *Eremurus persicus,* antibacterial activity, cytotoxic activity.

## INTRODUCTION

The genus *Eremurus* (Liliaceae) comprising nearly 50 species, is mainly restricted to central and western Asia (Chong et al., 2000) and six species are known to exist in Iran. *Eremurus persicus* locally called "Serish" is widely distributed in south, east and west of Iran. The roots are used as natural glue. The leaves are traditionally used to relieve constipation and treatment of diabetes, liver and stomach disorders. Polysaccharides have been reported as chemical constituents of species in the genus *Eremurus* (Chong et al., 2000). Since the literature survey revealed that there is no any information on the biological activities of *E. persicus*, we prompted to investi-gate the antibacterial activity of methanolic extract of flowering aerial parts of this plant by using the cup plate method and determine its cytotoxic effect after infection by two cell lines (HeLa and Caco-2) and further by MTT assay.

## MATERIALS AND METHODS

### Plant material

The flowering aerial part of *E. persicus* was collected from Golpayegan (Isfahan, Iran) in May 2010 and identified by Dr. Gh.R. Amin at the Pharmacognosy Department, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. A voucher specimen (NO. 197) has been deposited in the herbarium of the Department of Pharmacognosy, Pharmaceutical Sciences Branch, Islamic Azad University (IAU), Tehran, Iran.

### Extraction and isolation

The dried ground material was extracted by percolator apparatus

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**Table 1.** IC50 of the methanolic extract of *Eremurus persicus* and DMSO in cell lines.

| Cell line | *Eremurus persicus* extract IC50 (mg/ml) | DMSO 10% IC50 (v/v) |
|---|---|---|
| HeLa | 4.74 | 4.45 % |
| Caco-2 | 5 | 4.73 % |

using methanol (Merck). The extract was concentrated by rotary evaporator apparatus and the solvent removed to produce a dark brown gummy solid. The resulting extract was kept in a sterile vial in a dark and cool place for further tests.

**Bacterial strains**

Gram-positive bacteria including *Staphylococcus aureus* (PTCC 1431), *Staphylococcus epidermidis* (PTCC 1435) and *Bacillus cereus* (PTCC 1247), *Streptococcus pyogenes (*PTCC 1447), and Gram-negative bacteria including *Escherichia coli* (PTCC1399), *Salmonella typhi* (PTCC 1639), *Shigella dysantriae* (PTCC 1188*), Klebsiella pneumonia* (PTCC 1053*), Pseudomonas aeruginosa* (PTCC 1430) were obtained from Persian Type Culture Collection, Iranian Research Organization for Science and Technology (PTCC, Iran).

***Antibacterial assay***

Antibacterial activity of the methanolic crude extract of *E. persicus* was investigated against 9 bacterial strains by the cup plate method (Fazyl Bazzaz et al., 2005). An overnight bacterial culture containing 1.5 x $10^8$ CFU/ml was used to culture on surface of Muller -Hinton agar plates. The wells were made on agar plates. 1000, 500 and 250 mg, respectively, of the extract were dissolved in 1 ml DMSO 10% and then filtered. 80 l of each test solution was added to each well. Following diffusion of solutions into agar, the plates were incubated at 37°C for 24 h. The diameter of inhibition zones around each well were determined in comparison with the well of ciprofloxacin (31.25 mg/ml) which was used as positive control. A well added by 80 l DMSO 10% instead of the extract solution, served as negative control too. The experiments carried out 3 times and the results were presented as mean ± SD.

***Minimum inhibitory concentration (MIC)***

After confirmation of the antibacterial activity in methanolic crude extract of *E. persicus,* MIC of the extract was determine by testing 10 concentrations of the extract against sensitive Gram-positive and Gram-negative tested bacteria by the micro plate dilution method. The reconstituted extract was diluted to give concen-trations of 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.95, 0.97 and 0.48 mg/ml, respectively. The lowest concentration of the extract that could inhibit the bacterial growth was considered as MIC (Mehregan et al., 2008). The data was subject to statistical analysis of SPSS software.

**Cytotoxic activity**

***Cell culture***

HeLa (Human cervix carcinoma) and Caco-2 (Human colon carcinoma) cell lines were obtained from Iranian Cell Bank of Institute Pasteur of Iran. HeLa cells was grown in a flask and RPMI 1640 media (Gibco, Germany) which was supplemented with 10% fetal bovine serum; FBS (Gibco, Germany) and 20mg/ml gentamicin (Sigma) was added. In addition, Caco-2 cell line was grown in DMEM media (Gibco, Germany) which was supplemented with 20% FBS and 20 mg/ml gentamicin. All plated were incubated in a $CO_2$ incubator at 37°C. 100 µl of 3 x $10^5$ cells/ml suspension of HeLa cell line and 100 µl of 6 x $10^5$ cells/ml suspension of Caco-2 cell line transferred to a 96- well microplate and incubated at the same condition.

***MTT assay***

Based on determined Ic50 (Table 1), 17 mg methanolic extract of *E. persicus* dissolved in DMSO 1%, separately for HeLa and Caco-2 assay and 100 µl of each concentration was transferred in a row of separate microplate to make serial dilution. After 72 h incubation in a $CO_2$ incubator at 37ëC, 20 µl of MTT (5 mg/ml in PBS) was added and incubated at the same condition for 3 to 5 h for MTT assay. After Formazan crystallization, 50 µl of isobutyl alcohol was added and OD (Optical density) was read at 570 nm wave length (Figures 1 to 2).

## RESULTS

The inhibitory effects of methanolic extract of *E. persicus* against different test organisms are shown in Table 2 (the p-value mentioned in Table 2 is only for the 1000 mg/ml concentration of extract). This extract indicated significant antibacterial activity (growth inhibition zone diameters ranging from (12.5 to 32 mm) against some Gram-positive bacteria including *S. aureus, B. cereus, S. epidermidis* and Gram-negative bacteria including *E. coli*, *S. typhi* and specially *S. dysantriae*.

Also, the MIC of the extract was determined by using the micro plate dilution method for Gram-positive bacteria, *S. aureus* and *B. cereus.* In addition, the methanolic extract could inhibit the growth of other Gram-negative bacteria at 125 and 15.62 mg/ml, respectively; the extract could effectively inhibit other test bacteria, *E. coli, S. dysantriae* and *S. typhi*, even at concentrations as low as 125, 0.48 and 31.25 mg/ml, respectively.

The cytotoxicity effect of methanolic extract of *E. persicus on* HeLa cell and Caco-2 cell culture was evaluated by MTT assay. The result shows that the more concentration of extract in the cell culture the less OD was detected. It means, after increasing the concen-tration of methanolic extract the viability of cells were decreased. Similarly, both cell lines showed decrease in cell viability after the concentration of extract was increased and decrease in viability begins when 2.125 and 4.25 mg/ml of methanolic extract was added to

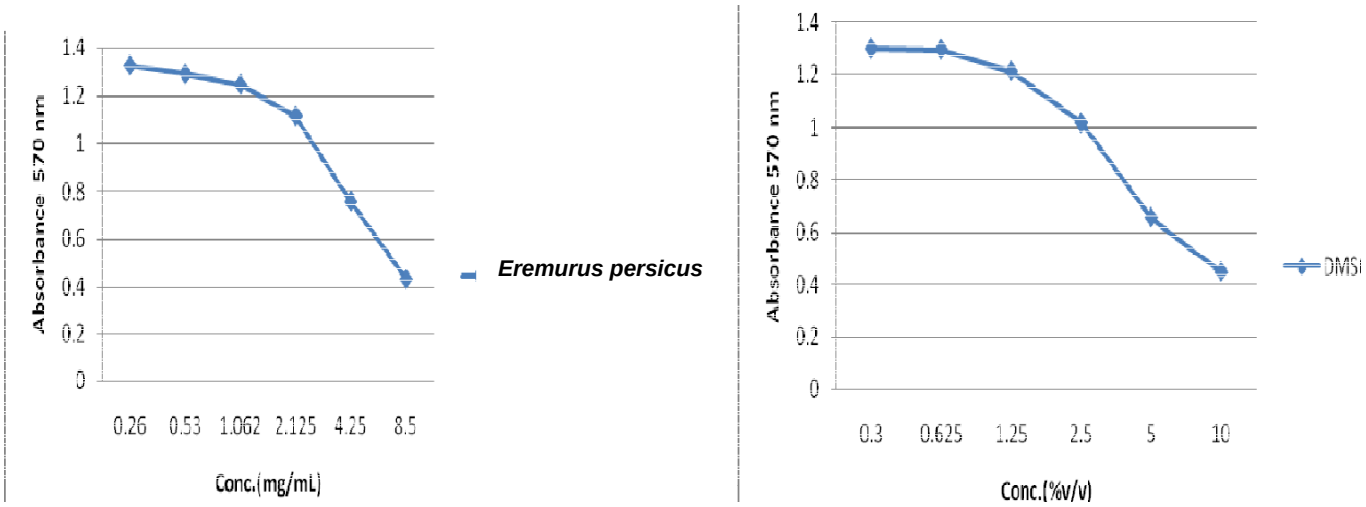


**Figure 1.** Absorbance value versus concentration of *E. persicus* extract and DMSO in Caco-2 cell line.

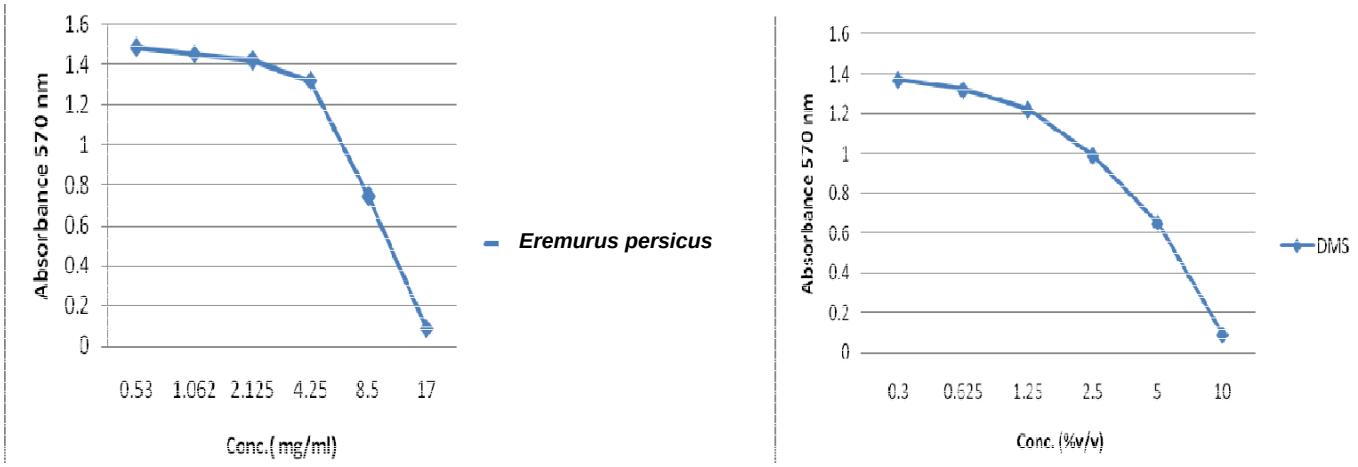


**Figure 2.** Absorbance value versus concentration of *E. persicus* extract and DMSO in HeLa cell line.

**Table 2.** Antibacterial activity of *E. persicus* methanolic extract against various bacteria, as obtained by cup plate method (n=6).

| S/No. | Organism | Mean zone of inhibition in mm and standard deviation (SD) | | | | P- value* | MIC(mg/ml) |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | | |
| | | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | | |
| 1 | *S. aureus* | 0.0±0.0 | 11.0±1.1 | 17.5±0.5 | 60.0±0.0 | <0.001 | 125 |
| 2 | *S. epidermidis* | 0.0±0.0 | 0.0±0.0 | 15.0±0.0 | 57.0±3.3 | <0.001 | resistance |
| 3 | *B. cereus* | 0.0±0.0 | 0.0±0.0 | 13.5±1.6 | 46.0±0.0 | <0.001 | 15.62 |
| 4 | *E-coli* | 0.0 ±0.0 | 0.0 ±0.0 | 12.5± 0.5 | 50.0±0.0 | <0.001 | 125 |
| 5 | *S. typhi* | 0.0±0.0 | 0.0 ±0.0 | 14.0±1.1 | 48.0±2.2 | <0.001 | 31.25 |
| 6 | *S. dysantriae* | 28.0±0.0 | 31.0±0.0 | 32.0±0.0 | 60.0±0.0 | <0.001 | 0.48 |

*t-test; A: Methanolic extract of *E. persicus* (250 mg/ml); B: Methanolic extract of *E. persicus* (500 mg/ml) ; C: Methanolic extract of *E. persicus* (1000 mg/ml) ; D: Ciprofloxacine (31.25 mg/ml).

Caco-2 and HeLa cells, respectively (Figures 3 to 4). In comparison of detected OD in both cell lines, HeLa cells

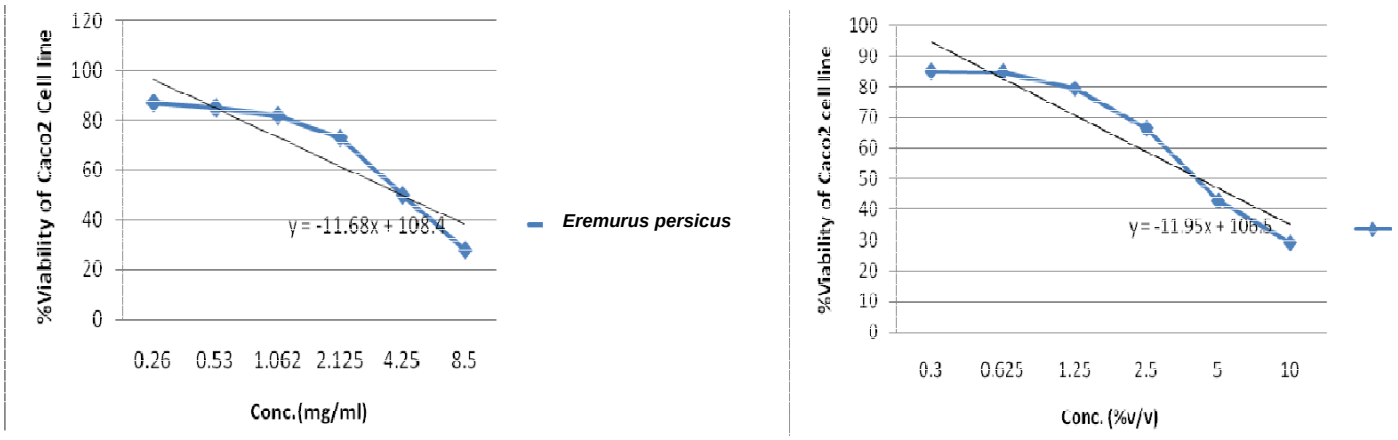


**Figure 3.** Effects of *E. persicus* extract and DMSO on cell viability in Caco-2 cell line.

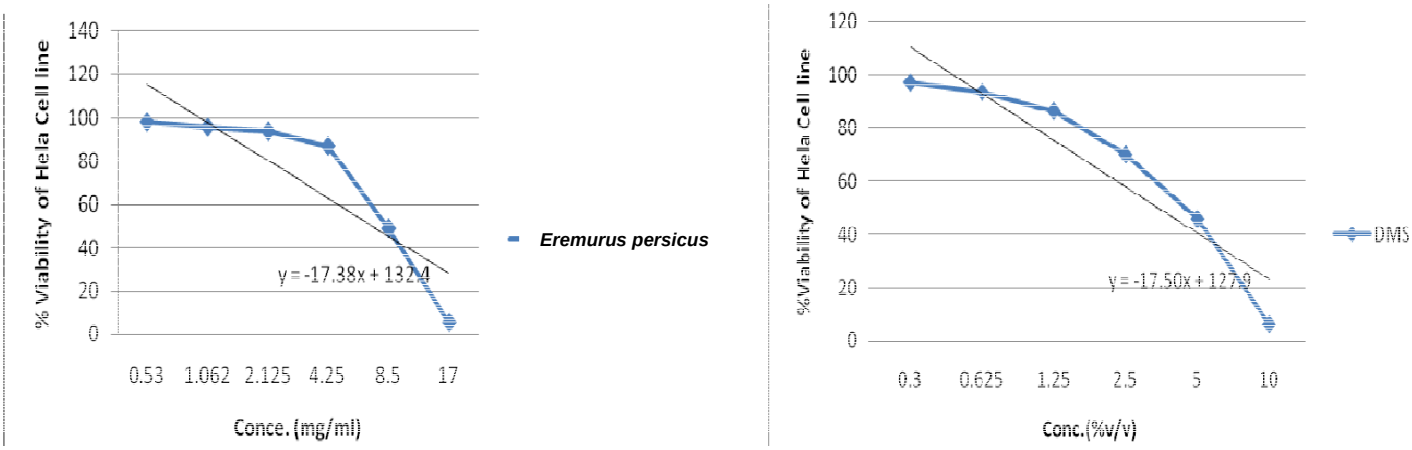


**Figure 4.** Effects of *E. persicus* extract and DMSO on cell viability in HeLa cell line.

shows more sensitivity than Caco-2 cell line against both *E. persicus* extract and DMSO concentrations as positive control.

## DISCUSSION

Since the presence of anthraquinones has been indicated in the aerial parts of other species of this genus (Chong et al., 2000), we suggest the strong antibacterial effect due to anthraquinones because they are more likely to possess antibacterial activity (Chukwujecwu et al., 2006). The cytotoxic activity is also related to the anthrax-quinones because it is shown that they are able to induce opoptosis due to the fact that they are suitable substrate for one-electron-reducing enzymes and effective redox cycler which lead to the production of oxygen derived free radicals that eventually induce opoptotic cell death (Kagedal et al., 1999). Based on the results of this study, further *in-vivo* and *ex-vivo* confirmatory tests are recommended.


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