



## Screening of biological activity of *Zosima absinthifolia* fruits extracts

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### Abstract

*Zosima absinthifolia* is a perennial herb which is distributed from Turkey to East Asia and Iran. Its fruits are used as a food spice in Iran and Turkey. In this work, we will study some biological activities of the fruits of the plant. The MTT assay indicated that methanol extract of the plant exhibited significant cytotoxic effects. In the DPPH assay, the extract showed high antioxidant potential with an  $RC_{50}$  value of  $143.5 \mu\text{g mL}^{-1}$ . In the disc diffusion assay, the methanol extract was found to have a significant antibacterial effects against *Bacillus cereus* and *Staphylococcus epidermidis*. Hexane, dichloromethane and methanol extracts displayed phytotoxic properties in the lettuce assay.

**Keywords:** Antimicrobial, antioxidant, cytotoxic, phytotoxic, *Zosima absinthifolia*.

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### INTRODUCTION

*Zosima absinthifolia* (Vent) Link. is a native species of Iran which belongs to the Apiaceae family. It is distributed from Turkey to Iran, Afghanistan and Central Asia and grows on the steppe, fields and lime stone slopes from an altitude of 400 to 2000 m (Davis 1972). The plant has been cultivated in Turkey and Iran for its aromatic seeds which are used as a condiment. Crushed seeds of the plant are used as a flavoring agent in seasoning, pickles and cookies. They are also used as a food spice in Iran where the plant is known as Golpar. Food spices derived from the Plants not only improve the flavor of foods but also have positive effects on our health. They may eliminate pathogenic microorganisms, act as a cancer chemo-preventive agent and may display many other biological activities causing they are used for treatment of some human ailments (Seideman 2005). Our previous study showed that the essential oil of the *Z. absinthifolia* seeds dominated by octyl acetate (87.4%), octyl octanoate (5.0%) and 1- octanol (2.3%) and exhibited high antibacterial effects against gram-positive bacteria like *Bacillus subtilis* and *Bacillus pumilus* (Razavi and Nejad-Ebrahimi 2009). The present study aims to investigate

some biological effects of the fruits extracts.

### MATERIAL AND METHODS

#### Plant materials

The seeds of *Z. absinthifolia* were collected from Vanyar in the East Azarbaijan province (Iran) in September 2007. A sample of this plant has been deposited at the herbarium for medicinal plants at the Faculty of Science of The University of Mohaghegh Ardabili (No:1387-1).

#### Plant extractions

The plant seeds were soxhlet extracted with n-hexane (Hex), dichloromethane (DCM) and methanol (Met), respectively. The extracts were concentrated in a rotary evaporator at 45°C for 1h and then dried in vacuum.

#### Cytotoxicity assay

Hela cell lines (Pasteur, C115) were grown in a RPMI 1640 (Gibco, No 51800-019) medium. Each 500 mL of the medium was supplemented with a 10% heat-inactivated fetal calf serum (FCS) in deionized water

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(Zhang et al. 2004). The stock solutions of the methanol extract of the *Z. absinthifolia* seeds were prepared by dissolving the extract in deionized water (100  $\mu$ L). The final concentrations of the extract were 0.10, 0.50, 0.75, 1 and 2 mg mL<sup>-1</sup>. Cells were plated in the appropriate media on 24-well microplates in a 500  $\mu$ L total volume at a density of  $6 \times 10^5$  cell mL<sup>-1</sup>. Triplicate wells were treated with media containing different concentrations of the extract. The plates were incubated at 37°C in 5% CO<sub>2</sub> for time course of 16 h. Cell viability was evaluated by the MTT colorimetric method (Doyle and Bryan 1999). The OD<sub>570</sub> was determined using a spectrophotometer. The 50% inhibitory concentration (IC<sub>50</sub>) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the control in the MTT assay. The Viability percentage was evaluated as OD<sub>treatment</sub>/OD<sub>control</sub> (Yeldjou et al. 2006).

#### Antioxidant assay

Serial dilutions were carried out with the stock solutions (1 mg mL<sup>-1</sup>) of the plant extracts to obtain concentrations of 0.5, 0.25, 0.175, 0.087, 0.043, 0.021, 0.010, .005, 0.002 and 0.001 mg mL<sup>-1</sup>. All of the solutions were prepared using methanol as solvent. Diluted solutions (5 mL each) were mixed with 5 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma) and allowed to stand for 3 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in duplicate and the average absorption was noted for each concentration. The RC<sub>50</sub> value, which is the concentration of the test material that reduced 50% of the free radical concentration, was calculated as mg mL<sup>-1</sup> (Razavi et al. 2008).

#### Antimicrobial assay

The antibacterial activity of the methanol extract were determined against *Bacillus subtilis* (PTCC 1207), *Bacillus cereus* (PTCC 1247), *Staphylococcus epidermidis* (PTCC 1114) and *Escherichia coli* (PTCC 1047) using the disc diffusion method. Muller-Hinton agar (MHA) (Oxoid) was used as bacterial medium. The filter paper discs (6 mm in diameter) were individually impregnated with 10  $\mu$ L of the stock solution of the extract (4 mg mL<sup>-1</sup>) and then placed onto the agar plates

which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37°C for 24 h. The diameters of inhibition zones were measured in millimeters. All the tests were performed in duplicate. Gentamicin (30  $\mu$ g) served as the positive control (Lorian 1996).

#### Phytotoxic assay

Lettuce (*Lactuca sativa* L. cv. *varamin*) seeds were used to test the germination response to different concentration of the plant fruit extracts. Hex and DCM extracts were dispersed as an emulsion in water using Tween 20. Four concentrations of the extracts (0.1, 1, 5 and 10 mg mL<sup>-1</sup>) were obtained by dilution of the emulsions with deionized water. The stock solution of Met extract was prepared using sterile water and different concentrations of the extract (0.1, 1, 5 and 10 mg mL<sup>-1</sup>) were obtained by dilution with deionized water. All seeds were surface sterilized with sodium hypo chloride (1%). Four replicates, with 25 seeds, were prepared for each treatment using sterile Petri dishes (90 mm) lined with one sterile filter paper (Whatman, number 2) and 5 mL of the different concentrations of the extracts was added to each Petri dish. Prepared plates were then placed in a germination cabinet at 25°C in the dark. After 1 week, in the each treatment, germination percentage was determined and root and shoot length was measured (Jefferson and Pennacchio 2003).

#### Statistical analysis

In all assays, SPSS 11.5 software was used for the statistical analysis. Analysis of variance (ANOVA) followed by the Duncan test was used to see the differences amongst the various groups. The significance level was set at  $p < 0.05$ .

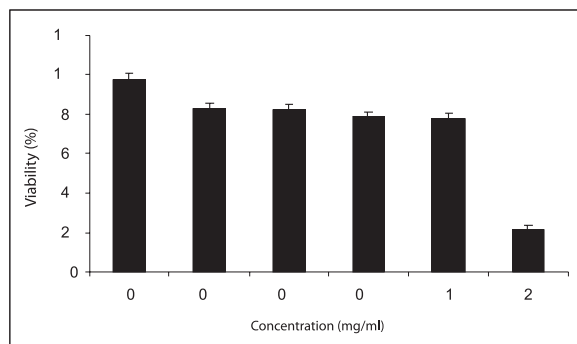
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## RESULTS AND DISCUSSION

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The results of the cytotoxic assay showed that the methanol extract of *Z. absinthifolia* fruits significantly exhibited cytotoxic properties against the Hela cell line. Table 1 presents the viability percentage of the Hela cell line treated with different concentration of the extract. By adding 2 mg mL<sup>-1</sup> of the extract, viability of cell line was reduced to 21.8%.

The DPPH assay indicated that methanol



**Fig. 1.** Effects of different concentration of methanol extract of *Zosima absinthifolia* fruits on viability of Hela cell line in MTT assay. Each bar represents the mean  $\pm$  SD.

extract of *Z. absinthifolia* fruits displayed high free radical scavenging activity with a  $RC_{50}$  value of  $143.5 \mu\text{g mL}^{-1}$ . The n-hexane and dichloromethane extracts exhibited modest antioxidant properties with a  $RC_{50}$  value of 631.9 and  $550.5 \mu\text{g mL}^{-1}$  (Table1).

The methanol extract of the plant fruits significantly showed antibacterial activity. The results from the disc diffusion method showed that *Bacillus cereus* is the most sensitive bacterium among tested bacteria with an inhibition zone of 15 mm. The extract indicated modest antibacterial activity against *Staphylococcus epidermidis* with an inhibition zone of 10.5 mm (Table 2).

According to the results given in Table3, all of the plant extracts significantly reduce seed germination and shoot and roots growth of lettuce in the phytotoxic assay. The  $10 \text{ mg mL}^{-1}$  of the DCM extract inhibited seed germination and root and shoot elongation of lettuce, approximately 30%, 30% and 10%, respectively.

AS mentioned before, the crushed fruits of the *Z. absinthifolia* are used as a food spice in Iran. Due to the antioxidant and cytotoxic potential of the plant fruits, besides being used as a seasoning, it could be used as a

**Table 1.** Antioxidant activity of the extracts of *Zosima absinthifolia* fruits.

Extracts	The DPPH assay ( $RC_{50}$ in $\mu\text{g mL}^{-1}$ )
Hexane	631.9
Dichloromethane	550.5
Methanol	143.5
Trolox (Control)	2.6

**Table 2.** Antimicrobial activity of methanol extract of *Zosima absinthifolia* fruits.

Microorganism	Extract ( $4 \text{ mg mL}^{-1}$ ) Inhibition zone (mm)	Gentamycin ( $30 \mu\text{g}$ ) Inhibition zone (mm)
<i>Bacillus subtilis</i>	8.4	31.5
<i>Bacillus cereus</i>	15.0	32.5
<i>Staphylococcus epidermidis</i>	10.5	33.0
<i>Escherichia coli</i>	7.5	21.3

chemo-preventive agent against cancer.

On the other hand, because of the antibacterial effects, using the plant fruits as a food additive can also prolong the shelf life of foods and cookies. It has been suggested that some synthetic food additives that are used as a preservative agent, convert ingested materials into toxic or carcinogenic substances. Thus, a novel way to reduce the proliferation of microorganisms in the foods is the use of natural antimicrobial agents.

It is stated in the literature that all parts of the plants of the *Zosima* genus are characterized by coumarin compounds like deltoin, marmesin, prangenin (Anonymous 2001). It is assumed that the biological effects of the extracts of the *Z. absinthifolia* fruits are related to coumarins. Although many coumarins have a clinical usage, there are a few reports about their unwanted side effects (Rahman 2000). Therefore, further research is need in order to obtain information regarding possible harmful effects of the *Z. absinthifolia* fruits on human health when they are used as a food additive.

The extracts of the *Z. absinthifolia* fruits were found to have modest to strong phytotoxic potential. The fruits are dispersing

**Table 3.** Phytotoxic activity of the hexane (Hex), dichloromethane (DCM) and methanol (Met) extracts of *Zosima absinthifolia* fruits.

Concentration ( $\text{mg mL}^{-1}$ )	Germination (%)			Shoot length (mm)			Root length (mm)		
	Hex	DCM	Met	Hex	DCM	Met	Hex	DCM	Met
0	$98 \pm 7.4 \text{ a}$	$92 \pm 5.3 \text{ a}$	$90 \pm 3.8 \text{ a}$	$16.5 \pm 1.8 \text{ a}$	$34.3 \pm 2.7 \text{ a}$	$20.4 \pm 1.1 \text{ a}$	$35.1 \pm 3.2 \text{ a}$	$24.5 \pm 3.3 \text{ a}$	$34.8 \pm 3.6 \text{ a}$
0.1	$80 \pm 3.1 \text{ b}$	$78 \pm 3.2 \text{ a}$	$76 \pm 2.2 \text{ a}$	$18.5 \pm 2.1 \text{ a}$	$41.9 \pm 3.3 \text{ a}$	$17.9 \pm 1.6 \text{ a}$	$30.1 \pm 4.8 \text{ a}$	$21.4 \pm 1.6 \text{ a}$	$29.1 \pm 3.6 \text{ a}$
1	$90 \pm 2.2 \text{ a}$	$74 \pm 1.4 \text{ a}$	$74 \pm 3.2 \text{ a}$	$10.0 \pm 1.1 \text{ bc}$	$33.5 \pm 2.5 \text{ a}$	$17.2 \pm 1.8 \text{ a}$	$35.8 \pm 4.1 \text{ a}$	$18.8 \pm 0.9 \text{ bc}$	$17.4 \pm 0.4 \text{ b}$
5	$82 \pm 3.3 \text{ b}$	$41 \pm 1.5 \text{ b}$	$59 \pm 4.5 \text{ b}$	$7.4 \pm 0.3 \text{ c}$	$5.9 \pm 2.2 \text{ b}$	$21.8 \pm 1.4 \text{ a}$	$27.7 \pm 3.8 \text{ a}$	$15.7 \pm 1.4 \text{ c}$	$9.8 \pm 1.2 \text{ bc}$
10	$77 \pm 4.4 \text{ c}$	$25 \pm 1.8 \text{ c}$	$42 \pm 3.6 \text{ c}$	$6.5 \pm 0.8 \text{ c}$	$4.5 \pm 0.8 \text{ b}$	$14.4 \pm 0.9 \text{ a}$	$23.6 \pm 4 \text{ a}$	$9.1 \pm 1.3 \text{ d}$	$5.0 \pm 0.9 \text{ c}$

Mean values in the same column followed by the same letter are not significantly different at the 0.05 level according to the Duncan test.

by the wind and could donate allelopathic properties to the plant. The allelopathy potential of plants has an important role in the plant productivity of the agroecosystems and could cause considerable resistance in plants against weeds, pathogens and herbivores.

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### REFERENCES

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- Anonymous (2001) DNP- CD-ROM Dictionary of natural products. Ver. 9:2, Chapman and Hall CRC, Florida.
- Davis PH (1972) Flora of Turkey. Edinburg University Press, Edinburg.
- Doyle A, Griffiths JB (1998) Cell and tissue culture (Laboratory procedures in biotechnology press). John Wiley Press, West Sussex.
- Jefferson LV, Pennacchio M (2003) Allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination. Journal of Arid Environments 55, 275-285.
- Lorian V (1996) Antibiotic in Laboratory Medicine. Williams & Wilkins, Philadelphia.
- Rahman AU (2000) Studies in natural product chemistry. Vol 23. Elsevier Science B.V., Amsterdam.
- Razavi SM, Nazemiyeh H, Hajiboland R, Kumaramasamy Y, Delazar A, Nahar L (2008) Coumarins from aerial parts of *Prangos uloptera*. Brazilian Journal of Pharmacognosy 18, 1-5.
- Razavi SM, Nejad-Ebrahimi S (2009) Chemical composition, allelopathic and antimicrobial potentials of the essential oil of *Zosima absinthifolia* (Vent) Link. fruits from Iran. Natural Product Research, In Press.
- Seidemann J (2005) World spice plants. Springer, Heidelberg.
- Yeldjou C, Moree P, Techounwou PB (2006) Dose and time-dependent response of human leukemia (HL-69) cells to Arsenic trioxide treatment. International Journal of Environmental Research and Public Health 3, 136-140.
- Zhang Q, Wu J, Hu Z, Li D (2004) Induction of HL-60 apoptosis by ethyl acetate extract of *Cratogeomys sinensis* fungal mycelium. Life Sciences 75, 2911-2919.

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## ***Zosima absinthifolia* Meyve Özütlendinde Biyolojik Aktivitenin Taranması**

### **Özet**

*Zosima absinthifolia* Türkiye'den Asyanin dogusu ve Iran'a kadar yayilis gösteren çok yıllık otsu bir bitkidir. Meyveleri Iran ve Türkiye'de baharat olarak kullanilir. Bu çalışmada bu bitkinin meyvelerinde bazı biyolojik aktiviteleri araştırılmıştır. MTT analizi bitkiden elde edilen metanol özütünün önemli sitotoksik etkiler göstermiştir. Özüt, DPPH analizinde ise 143.5 µg mL<sup>-1</sup> RC<sub>50</sub> degeri ile yüksek bir antioksidan potansiyel göstermiştir. Disk difüzyon analizinde, metanol özütünün *Bacillus cereus* ve *Staphylococcus epidermidis*'e karsi önemli antibakteriyel etkilerinin olduğu bulundu. Hekzan, diklorometan ve metanol özütleri, marul deneyinde fitotoksik özellikler göstermiştir.

**Anahtar Kelimeler:** Antimikrobiyal, antioksidant, fitotoksik, sitotoksik, *Zosima absinthifolia*.