ANTIOXIDANT ACTIVITIES AND CYTOTOXIC EFFECTS OF WHOLE PLANT AND ISOLATED CULTURE OF ARTEMISIA AUCHERI BOISS.

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INTRODUCTION

Artemisia aucheri belong to the Asteraceae family. The 500 species of Artemisia are mainly found in Asia, Europe and North America. They are mostly perennial herbs and dominating the vast steppe communities of Asia. 1 Artemisia has 34 species that are found wild all over Iran. 2 The species of Artemisia share the common morphological characters: Herbs or small shrubs, frequently aromatic, leave alternate, capitates small, usually pendent, racemose, paniculate or capitates inflorescences, rarely solitary. 3 Artemisia species are widely used in traditional medicine all over the world with different and well known therapeutic applications (stomach ache, diarrhea, parasitism, intestinal and bronchial infections, angina, wounds, pimples, colds and coughs). 4 They exhibit anti-inflammatory, antifungal and antioxidant activities. 5 According to literature, over 260 Artemisia species have been investigated to reveal that they contain many classes of secondary metabolites including terpenoids, flavonoids, coumarins, glycosides, sterols and polyacetylenes. 6, 7, 8 Selidokon et al. 9 isolated the essential oil of Artemisia aucheri by stem distillation. The main constituents of the oil (from plants collected in Semnan province, Iran) were verbenone (21.5%), camphor (21.0%), 1, 8-cineole (8.3%), and trans-verbenol (8.1%).

The in vivo Artemisia aucheri herbal extract effected on the leishmania major cutaneous infection in susceptible Balb/C mice. 10 The essential oils of aerial parts of Absinthium, Asantonicum and Aspicigera tested were found to be toxic to adults of Sitophilus granarius (L). The oils showed about 80-90% mortality of granary weevil, S. granarius a dose of 9ug/l air after 48 h of exposure. 11 The fumigant activity of some essential oil from Artemisia species have been evaluated against a number of stored product insects. Fumigant toxicity of the essential oils has been reported from Annona against Sitophilus oryzae. 12 and Atridentata Nutt. Against some stored grain insects. 13 Artemisia aucheri Boiss oil had fungicidal properties to stored insect, 14 and Asieberi oil to Sitophilus oryzae and Tribolium castaneum. 15, 16 Artemisia annua and Achillea mililfolium extracts possess anti-feedant and toxic effects on Pieris rapae and can inhibit growth through various metabolic processes. 17 Also the essential oils of Artemisia annua and Artemisia taschernieviana showed antimicrobial and antifungal activity. 16, 17

Plant cell cultures are an attractive alternative source to whole plant for the production of high value secondary metabolites. 18, 19 However, a considerable progress has been made to stimulate production and accumulation of secondary metabolites using plant cell cultures. 20, 21

The purpose of the present study was thus to compare the cytotoxicity activity of alcoholic extract of Artemisia aucheri and its isolated culture then antioxidant activity of Artemisia aucheri.

MATERIALS AND METHODS

Samples

The aerial parts of wild A. aucheri were harvested in July, 2011 between Sidabad and Sarbandan near the city of Tehran. The plants were identified kindly by Professor Mozaffarian, Research Institute of forests and Rangelands, Tehran, Iran. Seeds of A. aucheri were purchased from Pakanbazar Isfahan co.

Disinfection method

The seeds of Artemisia aucheri were washed with mild detergent in water, rinsed with running tap water for 1 h and surface sterilized by shaking in 70% ethanol for 1 min, rinsed with distilled water and then dipped into 1.5% sodium hypochloride for 10 min, finally followed by washing with sterile distilled water three times.

Isolated Culture

The seeds were grown on Murashige and Skoog (MS) 22 medium without any growth regulators. One month old in vitro germinated seedlings of Artemisia aucheri were used as a source of explants for initiation of callus cultures. All cultures were incubated at 28 ± 1 °C under darkness. Subculture was done every month. After 5 passages, callus tissue was air-dried at 70°C and extracted by methanol for 24h at room temperature (27 ± 2.0 °C). The extracts were filtered and then solvents by vacuum rotary evaporator to obtain crude methanol extract and stored at 4°C for further usage.

Preparation of plant extracts

The fresh parts of plant were washed enough with tap water then twice with distilled water dried in the oven at 50°C and slightly crushed by hand then with an electric blender. Samples (10 g of the dried plant material) were individually soaked in 100 ml methanol for 24h at room temperature (27 ± 2.0 °C), then filtered through sterile cotton till clear filtrate obtained. The solvents by vacuum rotary evaporator to obtain crude methanol extract.
Cytotoxicity assays

The extract acute toxicity (mean lethal concentration) \((L_C_{50})\) was evaluated using the brine shrimp \((Artemia franciscana)\) assay as described elsewhere.\(^{23,24}\) Briefly, 10 mg of dried brine shrimp eggs (Advanced Hatchery Technology, INC, USA) were deposited in fresh filtered seawater and allowed to hatch for 24 h. The assay was performed dissolving the extract of \(A. aucheri\). Ten larvae were put in 10 ml seawater containing the extract of \(A. aucheri\) at concentrations between 18 and 1000 \(\mu g/ml\). The \(L_C_{50}\) assay was done using three replicates for each concentration, and counting of dead organisms was carried out 24 h after exposure to the different extract concentrations. All the experiments were conducted by quadruplicates and \(L_C_{50}\) values as well as 95% confidence intervals were calculated using the probit method.\(^{25}\)

Determination of free radical scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the extracts.\(^{26}\) Different concentration of extract \((10, 50, 100, 150\) and \(200 \mu g/ml\) in 50% methanol solution) were prepared. The reaction assay contained 3 ml of 0.060 mM DPPH (Sigma Chemical Co. Louis, Mo, USA) in methanol and 200 \(\mu l\) of extract solution. Experiments were carried out in triplicates. After 30 minutes at room temperature, the Abs values were measured at 517 nm on a spectrophotometer. Gallic acid was as reference and 50% methanol solution was used as vehicle control. The ability to scavenge DPPH radical was calculated by the following equation:

\[
\text{DPPH radical scavenging activity (\%) = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100.}
\]

RESULTS

To determine the optimal medium for the growth of callus from seedling, three MS basal media supplemented with different concentrations of vitamins and combinations of growth regulators were used (Table 1).

<table>
<thead>
<tr>
<th>Media codes</th>
<th>Basal Medium</th>
<th>Pyridoxine- HCl (mg/l)</th>
<th>Nicotinic acid (mg/l)</th>
<th>Thiamine- HCl (mg/l)</th>
<th>IAA (mg/l)</th>
<th>NAA (mg/l)</th>
<th>BAP (mg/l)</th>
<th>Kin (mg/l)</th>
<th>2,4-D (mg/l)</th>
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<td>1.0</td>
<td>-----</td>
<td>-----</td>
<td>1.0</td>
<td>-----</td>
</tr>
<tr>
<td>B2</td>
<td>MS</td>
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<td>0.5</td>
<td>0.5</td>
<td>-----</td>
<td>0.5</td>
<td>3.0</td>
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<tr>
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<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>4.0</td>
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</table>

The results presented in (Figure 1) shows that the seedling explants grown on B2 gave the highest frequency of callus formation and hard but on D3 was soft and succulent. On A2, the seedling explants gave callus, but, within 2-3 weeks callus differentiation to shoots. The medium B2 showed significant superiority in frequencies of callus formation. The results presented in (Figure 1) shows that the seedling explants grown on B2 gave the highest frequency of callus formation and hard but on D3 was soft and succulent. On A2, the seedling explants gave callus, but, within 2-3 weeks callus differentiation to shoots. The medium B2 showed significant superiority in frequencies of callus formation.

Cytotoxicity of extracts against Artemia franciscana

In comparison with the \(L_C_{50}\) of the plant and callus extract based on the brine shrimp assay after 24 h are presented in (Figure 2, 3). Values varied between 18-1000 \(\mu g/ml\) and 35-2000 \(\mu g/ml\) for 24 h exposure, respectively. These results, although indicate that most tested plant extract can be considered cytotoxic (\(L_C_{50} < 35 \mu g/ml\)), but callus extract had no \(L_C_{50}\) value in our tested concentrations.
DPPH radical scavenging activity

The methanolic extract of *Artemisia aucheri* exhibited a significant dose dependent inhibition DPPH activity, with a 50% inhibition (IC₅₀) at a concentration of 190 µg/ml. This is equivalent to IC₅₀ of the standard Gallic acid (8.5µg/ml) for DPPH activity. These results demonstrated that *Artemisia aucheri* extract has weak inhibitory activity as compared with Gallic acid, against the DPPH radical (Figure 4).

**Fig 4:** Antioxidant activity measured by DPPH assay of methanol extract of *A.aucheri* at 517 nm as compared to gallic acid

**DISCUSSION**

Our findings show that the toxic effects of *A.aucheri* were clearly observed by LC₅₀ values after 24 h of exposure. Earlier reports on Artemisia clearly depict the potential of the species to have toxic properties against pests and animal. 15,27,28,29 Hence, this finding is consistent with earlier reports. In aerial parts of *Artemisia aucheri* have been found cyclic monoterpenes and monoterpene hydroperoxides.30 The insecticidal constituents of many plant extracts and essential oils of many plant extracts and essential oils are mainly monoterpenoids.31,32,33 Monoterpenoids are typically volatile and rather lipophilic compounds that can penetrate into animals rapidly and interfere with their physiological function.34 For that reason, secondary metabolites of plants play a major role in cytotoxic, and they function through toxicity.35

But isolated culture of *Artemisia aucheri* extract had no effective cytotoxic. Even the toxic effect of callus extract at higher concentrations was not observed by LC₅₀ values. This result was forecastable. Because cytotoxic activity of callus extracts of *Nerium oleander* was increased with callus subculturing (from 4th to 14th passages). 36 Also pointed out that that bioactivity is highly dependent on chemotype and type of extraction. 37

Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. The present study was conducted to investigate the antioxidant potential of *Artemisia aucheri*. Antioxidant activity of *Artemisia aucheri* extract has been revealed by free radical scavenging. The model DPPH provides a method to evaluate antioxidant activity in a relatively short time compared to the other methods. In our present study, *Artemisia aucheri* extract showed scavenging activity of DPPH radical, which may be attributable to its hydrogen donating ability.

**CONCLUSION**

The present study suggests that methanolic extracts of two tested *Artemisia aucheri* are a potential source of active natural substances which have natural cytotoxic effects on brine shrimp and is antioxidant. But cytotoxic effects was not noticeable in isolated culture extract.

Further study is in progress in order to increase with callus subculturing of this plant.

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