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Research Article

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

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ABSTRACT

Artemisia genus is an important medicinal plant in Iran. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases. The present study was undertaken to evaluate the antioxidant activity of methanolic extract of *Artemisia aucheri* and cytotoxic effects of methanolic extract between *Artemisia aucheri* and callus culture of *Artemisia aucheri.* The plant extract was tested for DPPH (2, 2-diphenyl, 2-picryl hydrazyl) radical scavenging assay. Cytotoxicity of extract was assessed using the brine shrimp assay. Callus induction was initiated from seedling on a Murashige and Skoog (MS) basal medium containing different concentrations of vitamins and combinations of growth regulators. Induced calli were subcultured on the same medium. The extract exhibited scavenging potential with IC_{50} (inhibition concentration 50) value at a concentration of 190 µg/ml.
The highest percentage of callus formation frequency was obtained from seedling explants grown on MS media supplemented with (0.5 mg/l) thiamine- HCl + (3 mg/l) 6BAP + (0.5 mg/l) NAA. The callus extract at higher concentrations didn't show by LC_{50} values. The plant extract showed high cytotoxicity (LC_{50} < 35 µg/ml) on the brine shrimp assay. This result showed that *Artemisia aucheri* might possess potential secondary metabolites that may be useful for cytotoxicity.

Keywords: *Artemisia aucheri*, *Artemia franciscana*, cytotoxic, DPPH (2, 2-diphenyl, 2-picryl hydrazyl), tissue culture

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). [4,5] They exhibit anti-inflammatory, antitumor, antioxidant, antispasmodic, antimicrobial, insecticidal, antimalarial, 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, coumaring, glycosides, sterols and polyacetylenes.[6] Sefidkon et al.[7] 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.[8] The essential oils of aerial parts of *A.absinthium, A.santonicum and A.spicigera* tested were found to be toxic to adults of *Sitophilus granarus* (L.). The oils showed about 80-90% mortality of granary weevil, *S. granarus*at a dose of 9µl/l air after 48 h of exposure. [9] 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 *A.annua* against *Sitophilus oryzae*, [10] and *A.tridentata* Nutt. Against some stored grain insects. [11] *Artemisia aucheri* Boiss oil had fumigant properties to stored insect, [12] and *A.sieberi* oil to *Sitophilus oryzae* and *Tribolium castaneum*. [13,14] *Artemisia annua* and *Achillea millefoliun* extracts prossess anti-feedant and toxic effects on *Pieris rapae* and can inhibit growth through various metabolic processes. [15] 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 metabolies 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, Teheran, Iran. Seeds of *A. aucheri* were purchased from Pakanbazr Isfahan co.

Disinfection method

The seeds of *Artemisia aucheri* were washed with mild detergent in water, rinsed with running tap water for 1h 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 methanolic 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 methanolic extract.

Cytotoxicity assays

The extract acute toxicity (mean lethal concentration) (LC_{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 µg/ml. The LC_{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 LC_{50} values as well as 95% confidence intervals were calculated using the probit method. [25]

Dtermination 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 µg/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 µl 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:

DPPH radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample})/(Abs_{control})] \times 100$.

RSULTS

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 1: list of ms media supplemented with different growth regulators and concentrations of vitamins used for *A.aucheri* callus cultures.

Media codes	Basal Medium	Pyridoxine- HCl (mg/l)	Nicotinic acid (mg/l)	Thiamine- HCl (mg/l)	IAA (mg/l)	NAA (mg/l)	BAP (mg/l)	Kin (mg/l)	2,4,D (mg/l)
A_2	MS	0.5	0.5	0.1	1.0	-----	----	1.0	----
B_2	MS	0.5	0.5	0.5	-----	0.5	3.0	-----	-----
D_3	MS	--------	1.0	2.0	-----	----	-----	-----	4.0

A B C

Fig 1. A-C.Induction of callus and shoot in *Artemisia aucheri*. (A) Induction of callus from seedling on MS medium containing 3.0 mg/l BAP+ 0.5 mg/l NAA + 0.5 mg/l thiamine- HCl ; (B) Induction of callus from seedling on MS medium containing 4.0 mg/l 2,4,D + 1 mg/l nicotinic acid + 2 mg/l thiamine- HCl; (C) Shoot formation from callus of seedling on MS medium containing 1 mg/l Kin + 1 mg/l IAA.

The results presented in (Figure 1) shows that the seedling explants grown on B_2 gave the highest frequency of callus formation and hard but on D_3 was soft and succulent. On A_2, the seedling explants gave callus, but, within 2-3 weeks callus differentiation to shoots. The medium B_2 showed significant superiority in frequencies of callus formation.

In comparison with the LC_{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 18-1000µg/ml and 35- 2000µg/ml for 24 h exposure, respectively. These results, although indicate that most tested plant extract can be considered cytotoxic (LC_{50} < 35 µg/ml), but callus extract had not LC_{50} value in our tested concentrations.

Cytotoxicity of extracts aginast Artemia franciscana

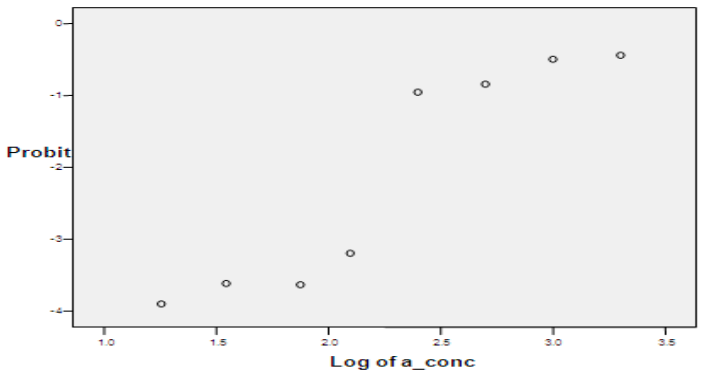

Fig 2:Regression line of mortality *Artemia franciscana* by callus extract of *A.aucheri*

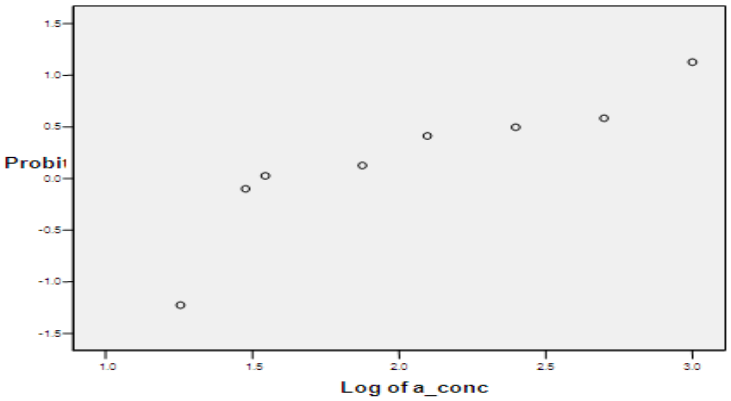

Fig 3:Regression line of mortality *Artemia franciscana* by *A.aucheri* extract

DPPH radical scavenging activity

The methanolic extract of *Artemisia aucheri* exhibited a significant dose dependent inhibition DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 190 µg/ml. This is equivalent to IC_{50} 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).

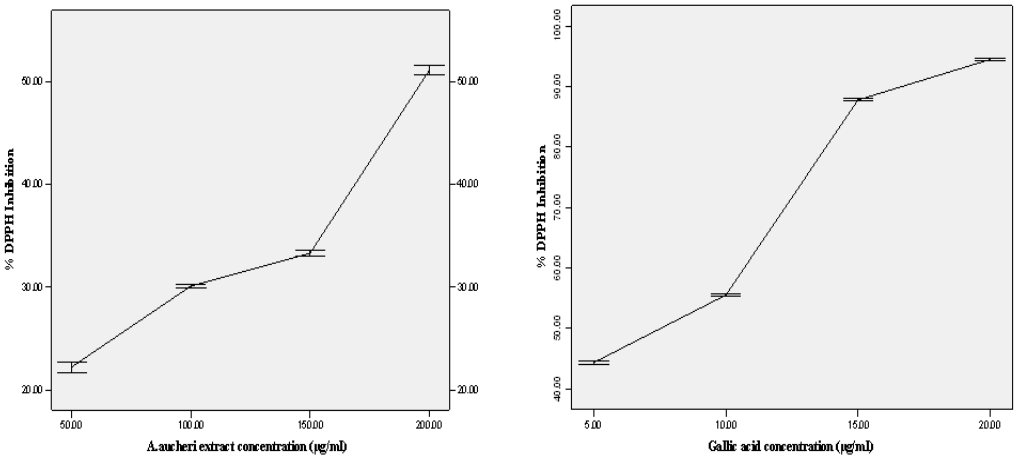

Fig 4 : Antioxidant activity measured by DPPH assay of methanol extract of *A.aucheri* at 517 nm as compared to gallic acid

DISCUSSION

Our findings show that the toxic effects of *A.aucheri* were clearly observed by LC_{50} 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 LC_{50} values. This reasult was forecastable. Becasue cytotoxic activity of callus extracts of *Nerium olender* 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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