Antioxidant Activity and Cytotoxicity of Dorema aucheri by Artemia urmiana: a Brine Shrimp Lethality Test

Ali Mirzaei1, Nooshin Mirzaei1, Mehdi Ghavamizadeh2*

1Medicinal Plant Research Center, Yasuj University of Medical sciences, Yasuj, Iran.
2*Student Research Committee, Yasuj University of Medical Sciences, Yasuj, Iran.
*Corresponding author: mehdighavamiz@gmail.com

Abstract: This study was designed for determination of phenolic contents, antioxidant activity and cytotoxic potential by Artemia urmiana system. Dorema aucheri is a wild vegetable and medicinal plant was collected in Yasuj, Iran and the samples were air dried for extraction. Material and methods: The cysts of A. urmiana were obtained from the Artemia Research Center (Urmia, Iran) and hatched in the laboratory for prepared of A. urmiana larve for study. Total phenolic, flavonoids levels and antioxidant activity were determined. Fresh larvae were exposed to different extracts and concentrations of D. aucheri for 24 hours and the numbers of live and dead larvae were estimated. LC50 of control and extracts were examined. The data were analyzed using the Finney’s probit analysis. Results: Hydro-alcoholic extract had the highest total phenol (72 mg Gallic acid/g extract) and flavonoids (12 mg Rutin/g extract) contents. The antioxidant activity measured by Diphenyl-pircylyhydrayzal (DPPH) (900 mmol trolox/g extract) and trolox equivalent antioxidant capacity (TEAC) (350 mmol trolox/g extract)methods. Hydro-alcohol extract displayed potent brine shrimp lethality with LC50 76.50±0.60μg.ml-1. Conclusion: Hydro alcohol extract was more lethal to larvae of A. urmiana compare to aqueous extract. So, Hydro alcohol extract could be a source of new compounds with biological activity. [Ali Mirzaei, Nooshin Mirzaei, Mehdi Ghavamizadeh, Antioxidant Activity and Cytotoxicity of Dorema aucheri by Artemia urmiana: a Brine Shrimp Lethality Test. Life Sci J 2013;10(12s):8-12](ISSN:1097-8135).

Key words: Artemia urmiana, Dorema aucheri, Cytotoxicity, Antioxidant activity, Total phenol.

Introduction

Since the ancient times, humans have used the plants in the treatment of various diseases. In modern medicine, herbal therapies due to their high level of interests and effectiveness have been taken high priority. So the popularity of herbal medicines is increasing globally. While, some synthetic drugs for the treatment of diseases have adverse effects (Mohamed Saleem et al., 1997). A few plants depending on species, genus and their grown source have therapeutic benefits. They constitute important compounds which called phytochemicals. Some phytochemicals compounds such as terpenoids, flavonoids, polyphenols, and alkaloids have significant antioxidant properties (Heber, 2004; Kaur et al., 2002) and could be useful but, some of them may be having potential toxicity and carcinogenic effect (Akinboro et al., 2007). Consuming of phytochemicals-rich foods is associated with low mortality in various diseases due to inhibiting the growth of tumors (Vecchia and Tavani, 1998). Many herbal preparations have a potent anti cancer activity against cancer cells (Lin et al., 1996). Plants – derived antioxidant such as flavonoids, carotenoids and terpenoids may block the function of many hormones and metabolic pathways in cancer cells (Caragay, 1992; Steinmetz et al., 1991). In some studies anti-tumor activity of quercetin derived from plant extracts have been reported (Craig, 2006).

In order to extract new anti-cancer agents from plant sources, it needed to screen tests using crude plant extracts. One of the most reliable screening methods is brine shrimp lethality test (BSLT). Brine shrimp is a species of aquatic crustaceans known as Artemia urmiana that belongs to the Artemidea family (Mirzaei et al., 2011). BSLT is very useful method for the detection of bioactive compounds in crude plant extracts. BSLT also has been used for toxicity screening of a variety of plant extracts, heavy metals, pesticides, food additives and drugs (Rezaeipoor-Kardost, 1996; Meyer et al., 1982). This method has been increasing globally due to lower costs, higher sensitivity for the detection of cytotoxic compounds and use smaller samples than other similar methods (Alluri, 2006). Regular cytotoxicity assays are costly and require discipline, equipment, blood collection and full-time personnel. BSLT was preferred due to 1-Artemia larvae similar with mammalian cells, 2-testing is quick, simple and cheap 3-use small amounts of material for testing 4-results due to the large number of larvae is closer to reality. Artemia urmiana belong to the small branchiopod crustacean Artemia is widely used in toxicology studies and laboratory bioassays (Sarabia et al., 2006). Accordingly, this study carried out for evaluation of cytotoxic effect of, Dorema aucheri extract on A. urmiana larvae. Dorema aucheri is a genus (family: Apiaceae) of plants which its...
consumption associate with hypocholesterolemia and hypotriglyceridemia (Hsiao et al., 2003). Its active ingredients was reported Senosionine, flavonoid and alkaloid compounds with antioxidant properties. (Wollenweber et al.,1995; Mirzaee et al., 2005).

Material and Methods

**Plant preparation and extraction**

*D. acheri* was collected from natural habitats in the flowering season in Yasuj, Iran. Plant sample was identified by a botanist and samples were dried in the shade. Herbarium specimens were kept at the plant medicine research center. For extraction maceration method used with two solvent systems, water and hydro alcohol for 48 hours at 40°C. The extracts were filtered with Whatman No.1 filter paper. Extracts was evaporated by a vacuum distillation system (Heidolph Laborota, model 4000; Germany). After concentration of extracts and weighted each of extracts, yield of each plant extract was determined.

**Hatching of Artemia urmiana cysts**

Artemia cysts were prepared from Artemia Research Center in Urmia, Iran. Cysts hatched in the laboratory by artificial sea water at 27–29 °C medium (Fig..1). During the test, the appropriate light, PH: 8.5, alkaline water with a salinity of 3% and temperature 28-30°C was regulated. After hatching, the larvae were, collected with a plastic pipette for LC50 study.

LC50 was estimated in five dilutions (250,500, 750, 1000 and 1500 µg/ml) of plant extracts at 24 hour.

In each plate 0.5ml of plant extract with different concentrations was added to 4.5 ml of the brine shrimp solution. Ten brine shrimp larvae which had developed for 48 hours were added to each plate. For each plant concentration, one control group was conducted which included 0.5 ml (vehicle treated, dimethyl sulfoxide (DMSO), with 4.5 mL of brine shrimp solution without extract. A positive control 2% Thymol was used also. The plates were kept covered with their lids in the darkness at room temperature for 24 hours. Feeding and air were not allowed in duration of study. After 24 hours, the number of dead and surviving larvae was counted on the plates then cytotoxicity of the extracts was determined. A percentage of deaths were calculated using the following formula and data were analyzed using probit analysis.

\[
\text{Death} \% = \frac{(d_{test} - d_{control})}{A_{control}} \times 100
\]

\[d_{test} = \text{the number of dead larvae in each test plate}\]

\[A_{control} = \text{the number of live larvae on control plates}\]

\[d_{control} = \text{the number of dead larvae in each control plate}\]

Each experiment was repeated three times. At duration of the each experiment, if the control group mortality was more than 10% experiment group. The procedure must be repeated (Hadjispyrou et al., 2001).

**In vitro antioxidant activity and phytochemical component assessment**

Major phytochemical compound e.g. total phenols (Folin and Ciocalteu, 1927) and total flavonoids (Zhishen et al., 1999) and antioxidant activity were estimated.

**Determination of total phenol**

The total phenolic contents of extracts were estimated using the Folin-Ciocalteau reagent with slight change. Total phenol was expressed as Gallic acid equivalent /g extract ( Karim, 2011).

**Determination of Total Flavonoid**

The total falavonoid level was measured with aluminium chloride (AlCl3) according to method of Kosalec et al. The total flavonoid values were determined in terms of rutin equivalents/g extract (Kosalec et al., 2004).

**Assessment of antioxidant activity**

The antioxidant activities of the extracts were tested using the following 2 assays: (1) 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Von Gadow et al., 1997), and (2) trolox equivalent antioxidant capacity (TEAC) radical methods (Re et al., 1999).

**Statistical Analysis**

LC50 values were determined using software Mini tab (ver.16) by Probit Analysis. To determine the difference between LC50 of different extract, t-student was used too. For Significant differences p<0.05 was applied.
Results

Aqueous and hydro alcohol extracts showed the lowest and highest total phenol values respectively. Total flavonoid content of extracts varied from 10 and 12 mg rutin as a standard in each gram of extracts (Fig. 2). The antioxidant activities of the extracts were determined as trolox equivalents (mMol trolox /g extract) using TEAC and DPPH assays. The highest antioxidant potential was observed with hydro alcohol extract in the DPPH (900 mMol trolox/g extract) and TEAC(350 mMol trolox/g extract) assays(Fig. 3). The aqueous extract showed the lowest activity with a values of 650 and 220 mMol trolox/g extract respectively. There was significant difference between the LC_{50} of aqueous and hydro alcoholic extracts of D. aucheri compared to control(\( p<0.05 \)). LC_{50} of hydro alcohol extracts of D. aucheri (76.50±0.6µg.ml^{-1}) was reported (Fig.4). The lethality rate was directly related to the extract concentration. LC_{50} of aqueous extract of D. aucheri (100.6±1.53µg.ml^{-1}) was reported also (Fig.5).

Discussion

BSLT is one of the most reliable tests that are very useful for the identification of bioactive compounds in crude plant extracts. This test has been used for toxicity screening of some plant extracts, heavy metals, pesticides, food additives and pharmaceutical compound. Due to its simplicity, high sensitivity and low costs has been received great attention to many researchers (Rezaeipoor-Kardost et al., 1996; Meyer et al., 1982). A. urmiana test also carried out for evaluation of plants potential that can be useful in the treatment of various diseases. This test, in addition to identifying toxic plants, is useful for identifying of potential therapeutic phytochemicals too. Plants contain phytochemicals that may be a good candidate for the treatment of various diseases. Although most plants are important sources of antioxidants, the toxicity of many plants have been reported due to phytochemical compounds (Rice-Evans et al., 2004). Hydro-alcohol extract of D. aucheri, have the highest levels of toxicity. Since the LC_{50} was less than 100 micrograms/ml of medium toxicity was considered. In this study in order to
determine the toxicity of medicinal plants *A. urmiana* was used, however in other studies *Artemia salina* has been used. According to the results, non-polar compounds have been extracted with ethanol solvents are more toxic than polar and hydrophilic compounds. According to experimental results of many researchers, compounds with LC₅₀ less than 30-1000 µg.ml⁻¹ were considered toxic (Wanyoike et al., 2004; Nakayoma and Yamada, 1995). Therefore *D. aucheri* was reported cytotoxic (Nakayoma and Yamada, 1995; Soliman et al., 2001). In this research and other researches, some compounds with LC₅₀ less than 100µg.ml⁻¹ were considered toxic. So, *D. aucheri* hydro alcohol extracts had the highest toxicity. According to present finding *D. aucheri* could be have high biological activity. It can be used for extract of toxic compounds with useful biological properties. The cytotoxic effects of phenol compounds, flavonoids and terpenoids, especially quercetin and rotenoid were reported in literature. In this study, the cytotoxicity of *D. aucheri* is consistent with traditional use. These results paralleled to other researcher experimental results (Lin et al., 1996; Soliman et al., 2001; Bidau et al., 2004). Further studies on *D.aucheri* cytotoxicity properties confirmed the results of this research. So cytotoxic effect of *D. aucheri* may be attributed to flavonoid compounds. According to the results of this study *D. aucheri* despite of a high biological potential. All extracts in this study were lethal to larvae of *A. urmiana* to varying degrees. Hence Hydroaloholic extract of *D. aucheri* has more toxic compared to aqueous extract. BSLT is a test system for toxicity screening of medicinal plants. For acquired of better results other systems such as Allium cepa and Ames test recommended.

**Conclusions**

In the present study *D. aucheri* was considered toxic. So it could be a source of new compounds with biological activity and its use should be done with caution and physician prescriptions.

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