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

Ali Mirzaei [1,] Nooshin Mirzaei[1], Mehdi Ghavamizadeh[2*]

[1]Medicinal 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. urmina* 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. LC $_{50}$ 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 Dipheny-picrylhydrazyl (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 LC_{50} 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). http://www.lifesciencesite.com. 2

Key words: *Artemia urmiana*, *Dorema auchri*, 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 has 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 (Vecchiaand 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 globallydue 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* larves 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: *Apiaccae*) 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. aucheri 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 larves were, collected with a plastic pipette for LC_{50} study.

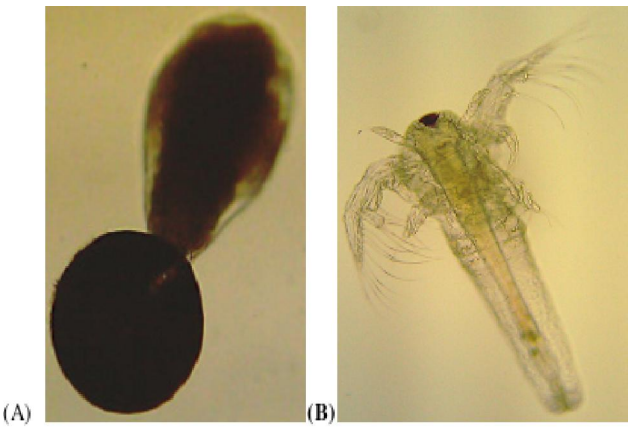

Fig. 1. Hatching of *Artemia urmiana* cysts. Recently hatched nauplius of Artemia uramiana. (A) and 48 growth (B) (Light microscopy ×40)

LC_{50} 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.

Death %=(d_{test}- $d_{control}$/$A_{control}$ ×100)

d_{test} = the number of dead larvae in each test plate

$A_{control}$= the number of live larvae on control plates

$d_{control}$= 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 Kosalecet 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-picrilhydrazyl (DPPH) radical (Von Gadow et al., 1997), and (2) trolox equivalent antioxidant capacity (TEAC) radical methods (Re et al., 1999).

Statistical Analysis

LC_{50} values were determined using software Mini tab (ver.16) by Probit Analysis. To determine the difference between LC_{50} 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).

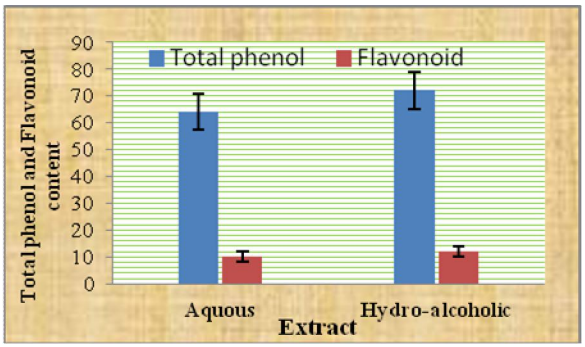

Fig. 2. Total phenol (mg Gallic Acid equivalent and flavonoid content (mg Rutin) of of *D. aucheri* in gram of extract

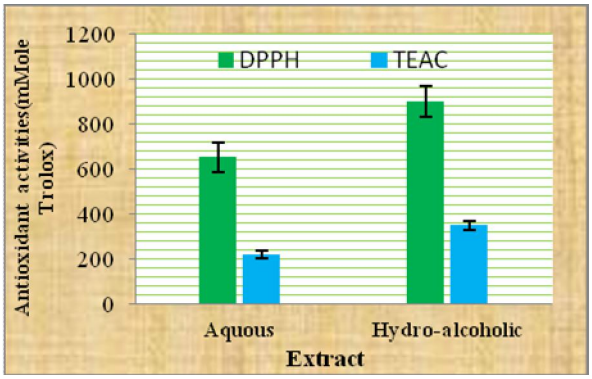

Fig. 3.The antioxidant activity of *D. aucheri* in terms *of* Dipheny-picrylhydrazyl (DPPH) andmMole trolox equivalent antioxidant capacity(TEAC).

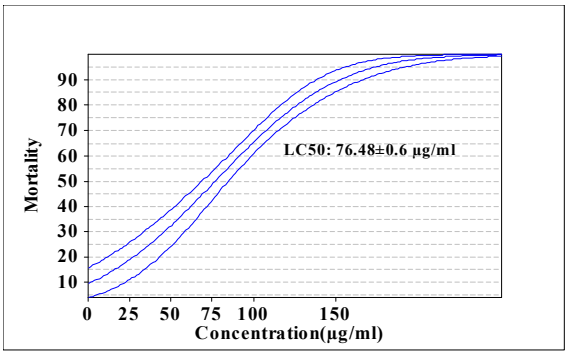

Fig. 4. LC_{50} of hydro alcohol extract of *D. aucheri* in different concentration by *Artemia urmiana:* a Brine Shrimp Lethality Test (BSLT).

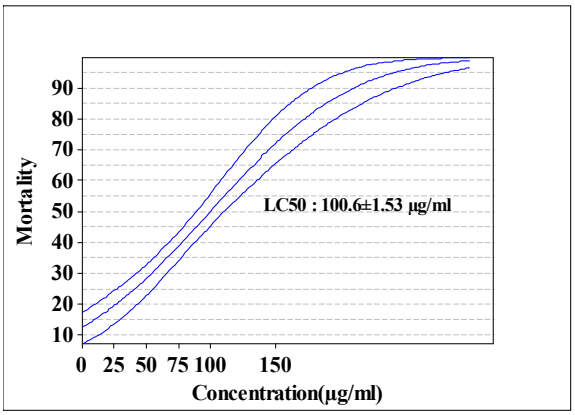

Fig. 5. LC_{50} of aqueous extract of *D.aucheri* in different concentration by *Artemia urmiana:* a Brine Shrimp Lethality Test (BSLT).

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 LC_{50} less than 30-1000 $\mu g.ml^{-1}$ 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 LC_{50} less than $100\mu g.ml^{-1}$ 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.

Acknowledgments

The authors thank to Medicinal Plant Research Center of Yasuj University of Medical Science for their financial supports.

References

1. Akinboro A, Bakare A A. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on Allium cepa Linn. Journal of Ethno pharmacology,, 2007;112(3): 470-475.
2. Alluri K, Tayi VNR, Dodda S, Mulabagal V, Hsin-Sheng T, Gottumukkala S. Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test). International Journal of Applied Science and Engineering, 2006; 4(2): 115-125.
3. American Cancer Society. A biotechnology company dedicated to cancer treatment, [viewed on 25 January]. Available at: <http:// www.cancervax. com/info/index.htm>.2006
4. Bidau CJ, Amat AG, Yajia M, Marti DA, Riglos AG, Silvestroni A. Evaluation of the genotoxicity of aqueous extracts of Ilex paraguariensis St. Hil. (Aquifoliaceae) using the Allium test Cytologia, 2004;69(2): 109–117.
5. Caragay AB.Cancer preventative foods and ingredients. *Technol,* 1992; 46: 65-68.
6. Craig, WJ.. Phytochemicals, guardians of our health. *J Am Diet Assoc,* 2006; 97: 199–S204.
7. Farzaneh M, Ahmadzadeh M, Hadian J, Tehrani A. Craig WJ. Phytochemicals: Guardians of our health. Chemical composition and antifungal activity of the essential oils of three species of arte Misa on som soil-born phytopathogens. Commun Agric Biol Sci, 2006; 71: 1327-33.
8. Hadjispyrou S, Kungolos A and Anagnostopoulos A.Toxicity bioaccumulation, and interactive effects of organotin,cadmium and chromium on Artermia franciscana. Ecotoxicology and Environmental Safety, 2001; 49: 179-186.
9. Heber D. Vegetables, fruits and phytoestrogens in the prevention of diseases. J. Postgrad Med, 2004;50(2): 145–149.
10. Hsiao G, Shen MY, Lin KH, Lan MH, Wu LY, Chou DS. Antioxidant and hepatoprotective effect of antrodia camphorata extract. J Agric Food Chem, 2003;51: 3302-3308.
11. Karim A., Sohail MN., Munir S and Sattar S.Pharmacology and phytochemistry of Pakistani herbs and herbal drugs used for treatment of diabetes., Int. J. Pharmacol. 2011; 7, 419-439.
12. Kaur C, Kapoor H. C.Antioxidants activity and total phenolic content of some Asian, vegetables. International Journal of Food Science and Technology, 2002;37: 153-161.
13. Khorsand-Mohammadpoor S, Yari M, Rustaiyan A, Masoudi S. Chemical constituents of the essential oil of Artemisia aucheri Boiss. a species endemic to Iran. J Essent Oil Res, 2002;14: 122–123.
14. Kosalec I., Bakmaz M., Pepeliniak S and Vladimir-Knezevic S.Quantitative analysis of the flavonoids in raw propolis from northern Croatia, Acta Pharm. 2004;54, 65-72.
15. LA Vecchia C, Tavani A.Fruits, vegetables and human cancer. Eur J Cancer, 1998; 7(1): 3–8.

16. Lin Y L, Juan I M, Chen Y L, Liang Y C and Lin J K. Composition of polyphenols in fresh tea leaves and associations of their oxygen-radial absorbing capacity with antiproliferative actions in fibroblast cells. J Agric Food Chem, 1996; 44: 1387–1394.
17. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nicholas DE and Mclaughlin JL.Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med, 1982; 45:31 – 34.
18. Mirzaee A, Hakimi MH, Sadeghi H.Total antioxidant activity and phenolic content of Dorema aucheri. Iranian J Biochem Mol Biol, 2005; 1(116).
19. Mirzaei M and Ali Mirzaei A.Comparison of the *Artemia salina* and *Artemia uramiana* bioassays for toxicity of 4 Iranian medicinal plants. *International Research Journal of Biological Sciences*. 2013; 2(3), 49-54.
20. Mohamed Saleem TS, Madhusudhana Chetty C, Mohsenzadeh S. Allelopathic effects of Artemisia on seed germination and seedling growth of Agropyron. Research and development 1997; (37): 62-67.
21. Mohsenzadeh, S.Allelopathic effects of Artemisia on seed germination and seedling growth of Agropyron.Research and development, 1997; 37: 62-67.
22. Nakayoma J and Yamada M.Suppression of active oxygen-indeed cytotoxicity by flavonoids.Biochem Pharmcol,1995;45:265-277.
23. Nemeth E, Bernath J.Biological activities of yarrow species (Achillea spp.). Current Pharmaceutical Design,2004;14(29):3151- 3167.
24. Ramkanth, V S T. Rajan, K. Mahesh Kumar, Gauthaman K. Hepatoprotective Herbs – A Review. Int J Res Pharm Sci, 2010;1(1): 1-5.
25. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice- Evans C,. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free. Radic. Biol. Med.1999; 26:1231-1237.
26. Rechinger KH.. Artemisia. In: Rechinger KH, Hedge IC(Eds), Flora Iranica. Compositae Akademische Druck and Verlagsanatalt, Graze, Austria, 1986; 158: 214.
27. Rezaeipoor-Kardost R. Cytikines and therapy. Fatemieh University of Medical Sciences. Iran.1996; 61 - 95.
28. Rice-Evans C. Flavonoids and isoflavones: absorption, metabolism and bioactivity. Free Rad Biol Med, 2004; 36: 827-838.
29. Shafizadeh F.Popular medicinal plants of Lorestan (Flora of Lorestan), 2001; 1: 82.
30. Soliman M I. Genotoxicity testing of neem plant (Azadirachta indica A. Juss.) using the Allium cepa chromosome aberration assay.Journal of Biological Sciences 20011;(11): 1021–10271-
31. Steinmetz KA and Potter JD.. Vegetables, fruits and cancer. I Epidemiology. Cancer Causes Control (Suppl.), 1991; 2: 325–357.
32. Von Gadow A., Joubert E., Hansmann CF. Comparison of antioxidant activity of aspalathin with that of other plant phenols of Rooibosed tea (Aspalathon linearis), α-tocopherol, BHT and BHA. J Agric. Food Chem. 1997; 45, 632-638.
33. Wanyoike GN, Chhabra CC, Lang'at- Thoruwa CC and Omar SA.Brine shrimp toxicity and antiplasmodial activity of five Kenyan medicinal plants. J. Ethnopharmacol, 2004; 90: 129 –133.
34. Wollenweber E, Dorr M, Rustayan A.Dorema aucheri, the first umbelliferous plant found to produce exudates flavonoids. Phytochem, 1995; 38: 1417-1427.

10/22/2013