

Alert for Consumption of *Dorema aucheri*: an Edible Medicinal Plant of Iran

Eftekhari M (Ph.D.)^{1,2}, Oskou F (Pharm.D.)¹, Tofighi Z (Ph.D.)^{2,3*}, Motevaseli E (Ph.D.)^{4*}, Jafari Nodooshan S (M.Sc.)⁴, Shams Ardekani MR (Ph.D.)¹

1- School of Pharmacy, International Campus, Tehran University of Medical Sciences, Tehran, Iran

2- Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, 14174-14411, Tehran, Iran

3- Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

4- Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

*Corresponding authors: Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14174-14411, Iran & Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran 14176-13151, Iran

Tel: +98-21-64121220, +98-21-43052000, Fax: +98-21-89771428

E-mail: ztofighi@tums.ac.ir, e_motevaseli@tums.ac.ir

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Abstract

Background: *Dorema aucheri* (Apiaceae) grows in west and southwest of Iran. Young leaves of this plant are used orally as pickle or for cooking some local foods, as well as for the treatment of parasitic digestive infections and constipation.

Objective: The aim of present study was evaluation of cytotoxic potential and total phenols determination of *D. aucheri* young aerial parts extract and fractions and its pickle extract.

Method: The dried powders of *D. aucheri* young aerial parts and pickles were macerated with 80% methanol and fractionated with petroleum ether (PE), chloroform (CL), ethyl acetate (EA) and methanol (ME). Cytotoxic activity of the total extract, fractions and pickle extract on HT-29, MDA-MB-231, A549, Hela cancer and normal fibroblast cell lines were assessed by MTT assay. Total phenol contents of all samples were determined by Folin-Ciocalteu method.

Results: The potent cytotoxic activities were shown by PE and CL fractions on normal fibroblast cell line with IC₅₀ equal to 17.66±2.3 and 48.80±1.24 µg/ml, respectively. Other fractions and extracts didn't have considerable cytotoxic effects on other cell lines. In addition, two mentioen fractions had the lowest total phenols. ME and EA fractions with highest amounts of phenols showed the lowest cytotoxicity.

Conclusion: It is concluded *D. aucheri* compounds have potential to exhibit toxic effects on healthy cells. On the other hand, there is negative correlation between cytotoxicity and phenol contents of *D. aucheri* fractions.

Keywords: Bilhar, Cytotoxic, MTT assay, Phenolics, Umbelliferae



Introduction

The *Dorema* genus belongs to Apiaceae family which consists six species in the flora of Iran [1]. *Dorema* genus produces an ammoniac gum resin which is known as “Oshagh” in Iranian traditional medicine [2]. The exudate with a warm and dry temperament (mizaj) has been applied for various disorders such as neurotic, urinary, gastrointestinal, respiratory and ophthalmic diseases [3-5].

Dorema aucheri is a perennial endemic species to Iran which grows in high mountains. This herb is locally known as “Bilhar” in coverage areas such as Kohgiluyeh and Boyer-Ahmad and Fars provinces in southwest of Iran [1].

The young aerial parts of the plant are used for pickling (Figure 1) as well as mixing in yogurt in west and southwest regions of Iran. Moreover in folk remedy, the herb is used for treatment of constipation, asthma, bronchitis

and parasites of digestive system [6, 7].

Previous pharmacological studies exhibited the extract of *D. aucheri* has hypoglycemic [8], anti-hyperlipidemic, anti-hypercholesterolamic [9], antimicrobial and antioxidant effects [10, 11]. Moreover, phytochemical investigation on this species showed the presence of methoxylated flavones such as salvigenin, nepetin, crisiliol and eupatorin [12]. Previous studies revealed that methylation of flavonoids causes’ significant cytotoxicity activity [13].

There are several reports on toxicity and cytotoxic activity of the plants extract [14-16]. Since young aerial parts of *D. aucheri* is used as food in some parts of Iran, the aims of present study was to evaluate cytotoxic activity of total extract and different fractions of dried powdered and pickled *D. aucheri* by MTT assay. In addition, total phenol contents of all mentioned samples were determined.



Figure 1- Pickle of *Dorema aucheri* aerial parts

Materials and Methods

Plant Collection

The young aerial parts of *D. aucheri* were collected from high level of Kohgiluyeh and Boyer-Ahmad Province, southwest of Iran, on March 2015. The plant was identified and authenticated by Professor F. Attar and deposited at Central Herbarium of Tehran University (No. 46056 TUH).

Extraction and fractionation

Dried powdered of aerial parts and pickled plant of *D. aucheri* extracted by 80% methanol at room temperature, separately. Both total and pickle extracts were concentrated and lyophilized. The total extract was fractionated with petroleum ether (PE), chloroform (CL), ethyl acetate (EA) and methanol (ME), successively via liquid-liquid extraction.

Evaluation of cytotoxic activity by MTT assay

Human cancer cell lines including HeLa cells (Cervical cancer), HT-29 (colon carcinoma), A549 (lung carcinoma) and MDA-MB-231 (breast cancer) and normal fibroblast cell line were purchased from Pasteur Institute, Tehran, Iran. The cells were cultured in RPMI 1640 medium (Biosera, England) supplemented with 10% FBS and 1% penicillin-streptomycin. The fibroblast cell line was maintained in Dulbecco's modified Eagle's medium (DMEM Ham's F12; PAA, Germany) supplemented with 15% FBS and 1% penicillin-streptomycin.

The cytotoxic activity of samples were assessed using MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide) (Sigma-Aldrich, USA) assay. After incubation

at 37°C, all of the cell lines were passaged using trypsinization and one hundred μL of the cells suspension in growth media were incubated into 96-well plates at density of 1×10^4 cells /well. After 24 h, one hundred μL of different concentrations of samples were added to each well. 48h later, 20 μL of MTT reagent (5 mg/ml PBS) were added per well and reincubated for 4 h. The medium were discharged and 100 μL of pure DMSO was added to each well. Finally, the absorbance was measured at 570 nm using a microplate reader (ELX800, BioTek, USA). The mixture of the medium and DMSO was used as negative control. All experiments were conducted in triplicate.

Cytotoxic activities of the samples were expressed as the concentration of sample that produces 50% of cell growth inhibition (IC_{50}) [17].

Selectivity indexes (SI) of various extracts and fractions were calculated by following formula:

$$\text{SI} = \frac{\text{IC}_{50} \text{ of sample against normal cells}}{\text{IC}_{50} \text{ of sample against cancer cells}} \text{ [18].}$$

Determination of total phenols

Phenolic contents of total and pickle extracts and all fractions were determined by Folin ciocalteau method [19]. In this method, phenols oxidation was occurred in alkaline solution have molybdotungstophosphoric heteropolyanion reagent with yellow color and resultant blue color of molybdotungstophosphate was measured. Maximum absorption of blue pigments depends on the phenols composition in the pH of solutions, obtained by sodium bicarbonate

or sodium carbonate [20]. The methanol solution (0.2 ml) of different concentration of each sample and gallic acid were added to 2 ml of 1:10 diluted Folin-Ciocalteu reagent (with distilled water). Five minutes later, 1.5 ml of saturated sodium bicarbonate solution (60 g/l) was added. Then, after 90 min, the absorption of the solutions were measured at 725 nm by spectrophotometer. The standard curve of gallic acid (GA) was prepared by various concentration of 0, 25, 50 and 100 mg/ml and total phenol contents were reported as GAE; Gallic Acid Equivalents (mg of gallic acid/ g of samples).

Statistical analysis

Results of cytotoxic activity and determination of total phenols were reported as the mean \pm SD of triplicate experiments and statistical analysis was done by Microsoft Excel 2013.

Results

Cytotoxic activity

As seen in Table 1, cytotoxic activity of total and pickle extracts, and different fractions of *D. aucheri* were investigated by MTT assay on HT-29, MDA-MB-231, A549 and Hela cancer cell lines and normal fibroblast cell line. Total extract, CL and PE fractions exhibited moderate cytotoxic activity against colorectal carcinoma with IC₅₀ equal to 184.72 \pm 4.90, 155.68 \pm 5.50 and 198.53 \pm 1.46 μ g/ml, respectively. IC₅₀ of other fractions and pickle extract were higher than 200 μ g/ml.

In breast cancer, CL and PE fractions demonstrated significant cytotoxic effect with IC₅₀ equal to 69.7 \pm 2.99 and 95.38 \pm 2.96 μ g/ml, respectively. Total extract and ME fraction had moderate cytotoxicity on MDA-MB-231 cell line with IC₅₀ equal to 133.98 \pm 0.01 and 177.41 \pm 2.63 μ g/ml, respectively. Pickle extract and EA fraction showed no cytotoxic effect.

Table 1- Cytotoxic activity of total extract, different fractions and pickle extract of *Dorema aucheri* aerial parts

Samples	IC ₅₀ (SI)					Total Phenols (GAE/ 100 g of sample)
	MDA-MB-231	A549	HT29	Hela	Fibroblast	
Total extract	133.98 \pm 0.007 (>1.49)	>200	184.72 \pm 4.90 (>1.08)	>200	>200	6.8 \pm 1.75
PE fraction	95.38 \pm 2.96 (0.18)	>200	198.53 \pm 1.46 (0.08)	117.66 \pm 3.01 (0.14)	17.00 \pm 2.30	2.8 \pm 0.22
CL fraction	69.7 \pm 2.99 (0.7)	147.66 \pm 0.23 (0.33)	155.68 \pm 5.50 (0.31)	145.38 \pm 0.10 (0.33)	48.84 \pm 1.24	5.2 \pm 0.11
EA fraction	>200	>200	>200	>200	>200	7.7 \pm 0.33
ME fraction	177.41 \pm 2.63 (>1.13)	>200	>200	>200	>200	12.4 \pm 0.76
Pickle extract	>200	171.27 \pm 1.9 (>1.17)	>200	>200	>200	3.3 \pm 0.22

Results are expressed as IC₅₀ value (μ g/ml), mean of three determinations \pm standard deviation. PE: Petroleum ether, CL: Chloroform, EA: Ethyl acetate, ME: methanol fraction; SI: selectivity index; HT-29: colorectal, MDA-MB-231: breast, A549: lung, Hela: cervical cancer cell lines

In lung cancer, pickle extract and CL fraction demonstrated moderate cytotoxicity with IC_{50} equal to 171.27 ± 1.9 and 147.66 ± 0.23 $\mu\text{g/ml}$, respectively. There were no cytotoxicity observed from other fractions and total extract.

In cervical cancer, only PE and CL fractions demonstrated moderate toxicity with IC_{50} equal to 117.66 ± 3.01 and 145.38 ± 0.10 $\mu\text{g/ml}$, respectively and other fractions and extracts showed no cytotoxicity.

The potent cytotoxic activity were shown by PE and CL fractions on normal fibroblast cell line with IC_{50} equal to 17.66 ± 2.3 and 48.8 ± 1.24 $\mu\text{g/ml}$, respectively. Other fractions and total extract didn't have any cytotoxic effect on normal cells.

Total Phenol Assay

Total phenol contents of total and pickle extracts and all fractions of *D. aucheri* were calculated based on $y = 0.0065x + 0.0337$, $r^2 = 0.998$, and reported in Table 1.

It was demonstrated that PE and CL fractions had the lowest amounts of phenols among fractions while ME had the highest phenol contents. The phenol contents of pickle extract was decreased about half of total extract.

Discussion

All over the world, people used traditional and folklore medicinal plants or herbal drugs in their health care or nutrition programs. The natural consumers believed that the mentioned products are safe but limited researches confirmed health hazards of some considered safe herbal remedies over the previous decades. Recent evidences had shown they

could be potentially toxic, carcinogenic and mutagenic [21, 22]. Poisoning from herbal drugs or natural products is often a consequence of incorrect identification and inappropriate preparation or administration, frequently self-administration of them [23, 24].

By technology progress, researchers could assay potential hazardous of herbal medicines. The present study evaluated cytotoxic activity of total extract and different fractions of dried powdered and pickled young aerial parts of *Dorema aucheri*, which was used as food in west and southwest of Iran.

According to the standard of US National Cancer institute (NCI), herbal extracts with IC_{50} lower than 20 $\mu\text{g/ml}$ were considered as cytotoxic on cancer cells [25]. Following this criterion, potent cytotoxic effects of PE and CL fractions on normal fibroblast cell line were confirmed which mentioned there are toxic compounds in plant which effect on healthy cells. In addition, the amount of selectivity index (SI) showed selective or non-selective category of cytotoxic compounds. If SI was more than 10, the cytotoxicity was selective while compounds with SIs between 1-10 were classified as non-selective [25]. In current research, none of extracts and fractions demonstrated selective manner.

A previous study showed methanol extract of aerial parts of *D. aucheri* had considerable cytotoxic effects on HepG2 and A549 cell lines with IC_{50} equal to 20.09 and 48.65 $\mu\text{g/ml}$, respectively [6]. However, the present study demonstrated total extract and fractions of *D. aucheri* had no considerable cytotoxicity against A549 lung cancer cell line. Also, there were reports about *D. aucheri* abilities to

induce necrosis and tumorigenic effects in rats. It was exhibited *D. aucheri* extract in dose of 400 mg/kg could increase the breast tumor volumes of rats in comparison to groups receiving only DMBA. It was noticeable by consumption of 200 mg/kg of extract, tumor tissue necrosis was observed [26]. Another study demonstrated injection of *D. aucheri* extract to healthy mice had induced hepatotoxicity as necrosis, inflammation, liver cell proliferation, cholestasis and increase of liver enzymes and bilirubin in a dose dependent manner [16]. Our cytotoxicity results on healthy cells confirmed these reports.

Phenolic compounds from different structures play an important role in fighting against cancers in *invitro* and *invivo* models [27]. Present study demonstrated ME and EA fractions of *D. aucheri* with highest amounts of phenols had not considerable cytotoxicity, while PE and CL fractions with lowest amounts of phenolic compounds demonstrated potent toxicity.

Conclusion

In conclusion, PE and CL fractions of *D. aucheri* extract demonstrated significant cytotoxic effects on breast and normal cell

lines. It is considerable that cytotoxicity of both mentioned fractions on normal cells were higher than cancer cell lines, therefore usage of them in chemotherapy were not appropriated because of their side effects, patient compliance and other factors. The cytotoxic effect of *D. aucheri* on normal cells confirmed that the phytochemical compounds of this plant can induce cancer in healthy persons. Since the aerial parts and pickle of this plant were used as local food in some Provinces of Iran, more studies are proposed on investigation of cytotoxic compounds, evaluation of acute and sub chronic toxicity of plant and its pickle.

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Conflict of interest

The authors declare that there is no conflict of interest

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