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In vitro Cytotoxic Activity of the Essential Oil of *Dorema ammoniacum* D. Don.

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Abstract: *Dorema ammoniacum* is a perennial herb which its gum resin is used in traditional medicine of Iran and also widely used in food, cosmetic and detergent industries.In the present study, *in vitro* cytotoxic activity of the essential oil from ripe fruits of *D. ammoniacum* was investigated against two human cancer (MCF and SW480) and two normal (HFSF and HFLP) cell lines. Ripe fruits of *D. ammoniacum* collected just in deciduous time were subjected to hydro-distillation to yield essential oil which was subsequently analyzed by GC and GC/MS. MTT assay used for cytotoxic activity.Among four cell lines tested SW480 and, MCF-7 are cancer lines and were more sensitive to *D. ammoniacum* oil than other two normal cell lines. On the basis of these results the essential oil *D. ammoniacum* has antimicrobial activity and low cytotoxicity activity provide a scientific basis for the traditional use.

Key words: *Dorema ammoniacum* · Apiaceae · Essential oil · Cytotoxicity · MTT assay

INTRODUCTION

The essential oils of aromatic plants and their components have a wide range of applications in ethno-medicine, preservation, food flavoring and fragrances and in the perfume industries [1]. Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics [2]. The genus *Dorema* D. Don (Apiaceae) is represented in the flora of Iran by six species, among which two are endemic as *D. ammoniacum* D. Don. and *D. aucheri* Boiss. are endemic [3]. *D. ammoniacum*, a vulnerable species, grows to height about 1-2 m and in spring and early summer contains a milky juice. It is one of the most important endemic medicinal plants in many arid and semi-arid regions of Iran such as Yazd, Isfahan and Semnan provinces which known with local Persian names of Kandal, Vasha or Koma-kandal [3-4]. *D. ammoniacum* produces a medicinal gum resin commonly known as Ammoniacum or gum ammoniac is found in cavities in stems, roots and petioles [5]. Resin exudes from punctures in the stem, which can occur from insect attack. The resin serves as a carminative, diaphoretic, mildly diuretic, expectorant, poultice, stimulant, antimicrobial and vasodilator [6]. It is still used in Indian and Western Medicine and is listed in the British pharmacopoeia as an antispasmodic and expectorant. It is occasionally used for chronic bronchitis and persistent coughs [5]. Antimicrobial activity of the dichloromethane-methanol (1:1) extract of the plant gum has previously been reported [7]. The literature survey revealed that the essential oil composition of *D. aucheri* aerial parts [8] and *D. ammoniacum* leaves [9] have been previously reported, but the chemical composition and cytotoxicity of the

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essential oils of *D. ammoniacum* fruits have not been investigated to date and hence we focused our attention on the present study for possible uses of its oil in pharmacy systems which has not been investigated previously. In previous our study, *In vitro* antimicrobial activity of the oil was evaluated against seven Gram-positive and Gram-negative bacteria (*Bacillus subtilis, Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and three fungi (*Candida albicans, Saccharomyces cerevisiae* and *Aspergillus niger*). The results of antimicrobial assay of the oil by disc diffusion method and MIC values indicated that the oil exhibited moderate to high antimicrobial activity especially against *B. subtilis and S. epidermidis* with MIC value of 3.75 mg/ml [10].

MATERIALS AND METHODS

Plant Material: Ripe fruits of *D. ammoniacum* were collected just in deciduous time from Semnan road toward Firoozkuh after Bashm defile (35° 46' 11? N, 52° 52' 38? E and altitude of 1300 m), Semnan province, Iran. A voucher specimen (AS-85406) has been deposited at the Herbarium of Ecology and Systematic Department, Research Institute of Applied Science, Shahid Beheshti University, Tehran, Iran.

Isolation of Essential Oil: Dried fruits of the plant (500 g) were hydrodistilled for 3 hours, using a Clevenger-type apparatus to yield 0.6.% (w/w) of yellowish oil. After decanting and drying of the oil over anhydrous sodium sulfate, was stored at low temperature before analysis.

GC and GC-MS Analyses: GC analysis of the oil was coducted using a Themoquest-Finnigan instrument equipped with a DB-5 fused silica column (30 m × 0.25 µm). Nitrogen was used as the carrier gas at the constant flow of 1.1 ml/min. The oven temperature was held at 60°C for one min, then programmed to 250°C at a rate of 4°C/min and then held for 10 min. The injector and detector (FID) temperature were kept 250°C and 280°C, respectively. The split ratio was 1.50. GC-MS analysis was carried out on a Themoquest-Finnigan Trace GC/MS instrument equipped with a DB-5 fused silica column (30 m × 0.25 µm). The oven temperature was raised from 60°C to 250°C at a rate of 4°C/min and then held 250°C for 10 min; transfer line temperature was scanned over the 45-465 amu with an ionizing voltage of 70 eV and ionization current of 150 µA.

Identification of the Oil Components: The constituents of the essential oil were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkenes (C_6-C_{24}) and the oil on DB-5 column under the same conditions. The components of the essential oil were identified by comparision of their mass spectra with those of a computer library or with authentic samples and confirmed by comparision of their retention indices, either with data published in the literature [11].

Cell Line and Cultures: The human fetal skin fibroblast (HFSF), Human colon adenocarcinoma cell line (SW480), Human breast cancer cell lines (MCF) and human fetal liver fibroblast (HFLP) were obtained from Pasteur Institute of Iran. The cells grown in RPMI 1640 supplemented with 10% fetal calf serum, 1% glutamine, 100 U/ml penicillin/streptomycin and 0.5 µg/ml fondison. Cells were cultured in a humidified atmosphere at 37 °C in CO_2.

Cytotoxicity Assay: The assay detects the reduction of MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] by mitochondrial dehydrogenase to blue formazan product, which reflects the normal functioning of mitochondrial and cell viability [12]. Briefly, the cells (5×10^4) were seeded in each well containing 100µl of the RPMI medium supplemented with 10% FBS in a 96-well plate. Cells were permitted to adhere for 24 h and then treated with essential oils in medium for 24 h, 20µl of 5 mg/ml MTT in phosphate buffered saline (PBS) was added to each well and the plate was incubated at 37 °C for 4 h. The medium was removed, 100µl of dimethyl sulfoxide (DMSO) was then added to each well. The optical density was measured at 490 nm. The cytotoxicity was calculated after comparing with the control (treated with 0.1% DMSO). Cytotoxicity is expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50}). All tests and analysis were run in triplicate and mean values recorded.

Statistics: All the experimental data for cytotoxic activity are presented as mean ± SEM of 3 individual samples.The statistical analysis was performed with Statgraphics (Centurion XV) and Excel software. The multi-factorial

ANOVA analysis followed by the Tukey multiple comparison tests were used for statistical comparisons. A P-value of less than 0.05 was assumed for significant differences.

RESULTS AND DISCUSSION

Essential Oil Analysis: The hydrodistillation of *D. ammoniacum* fruits gave a yellow oil in 0.09% (w/w) yield, based on the dry weight of the fruit. Twenty-nine components were identified representing 95.1% of the total oil. The qualitative and quantitative essential oil compositions are presented in table 1, whereas compounds were listed in order of their elution on the DB-5 column. The major constituents of the oil were (*Z*)-ocimenone (22.3%), (*E*)-ocimenone (18.1%) and *β*-cyclocitral (9.9%). (*Z*)- and (*E*)-ocimenone have been previously reported as the main compounds of the essential oil of *Ferula latisecta* [13] The classification of the identified compounds, based on major groups is summarized at the end of table 1 and shows that oxygenated monoterpenes (58.4%) were the main group of compounds. In an earlier investigation on the essential oil composition of *D. ammoniacum* leaves [9] *α*-gurjunene (49.5%), *β*-gurjunene (19.0%) and *α*-selinene (4.6%) were found to be the main constituents while, in our study (*Z*)- and (*E*)-ocimenone, *β*-cyclocitral and *ar*-curcumene were characterized as the major components, which could be attributed to their ecological variability or plant part. Masoudi *et al*, have been reported α-eudesmol (31.2%) and δ-cadinene (10.9%) as the main components of the essential oil of *D. aucheri* [8].

Cytotoxicity: Essential oils have been shown to exhibit cytotoxic activity generally without being mutagenic in various organisms [1]. Since the differential cytotoxicity is also a useful feature for potential antitumor agents, the cytotoxic activity of the essential oil from *D. ammoniacum* was evaluated in four cancer and normal cell line by MTT assay based on cell viability. Human fetal skin fibroblast (HFSF), Human colon adenocarcinoma cell line (SW480), Human breast cancer cell lines (MCF) and human fetal liver fibroblast (HFLP) cells were exposed to the oil at dosages from 4.8 to 2500µg/ml (table 2). The results of cytotoxicity of the essential oil of *D. ammoniacum* on four cell line were shown in table 2. Among four cell lines tested, SW480 and MCF-7 are cancer lines and with IC_{50} value 625 and 312, respectively, showed were more sensitive to *D. ammoniacum* oil than other two fibroblast cell lines.

Table 1: Essential oil composition of *Dorema ammoniacum* fruits

No.	Compound	RI*	%
1	1,3,8-*p*-Menthatriene	1119	0.5
2	(*E*)-Tagetone	1126	2.2
3	(*Z*)-Tagetone	1133	3.2
4	(*E*)-5-undecen-3-yne	1163	0.7
5	*trans*-2-Caren-4-ol	1178	2.2
6	*β*-Cyclocitral	1189	9.9
7	(*Z*)-Ocimenone	1213	22.3
8	(*E*)-Ocimenone	1220	18.1
9	*p*-Mentha-1,8-diene	1246	0.5
10	Piperitenone oxide	1293	0.5
11	*α*-Cubebene	1356	0.4
12	*α*-Copaene	1385	3.2
13	*β*-Bourbonene	1395	4.1
14	Italicene	1414	1.0
15	di-epi-*α*-Cedrene	1427	2.9
16	α-Longipinene	1430	0.9
17	*β*-Cedrene	1433	0.5
18	*β*-Barbatene	1457	3.0
19	*α*-Humulene	1462	1.1
20	*ar*-Curcumene	1475	6.4
21	(*Z*)-(*E*)-Farnesene	1481	0.6
22	Germacrene D	1487	3.0
23	Bicyclogermacrene	1502	0.9
24	Cuparene	1506	2.9
25	*δ*-Cadinene	1522	0.8
26	Spathulenol	1576	1.2
27	Caryophyllene oxide	1583	0.7
28	Heptadecanoic acid	2069	1.4
	Monoterpene hydrocarbons		1
	Oxygenated monoterpenes		58.4
	Sesquiterpene hydrocarbons		31.7
	Oxygenated sesquiterpenes		1.9
	Others		2.1
	Total identified		95.1

* RI: Retention indices relative to C_6–C_{24} *n*-alkanes on DB-5 column

Table 2: cytotoxicity of the essential oil from *Dorema ammoniacum*

Cell lines	IC_{50} (µg/ml)[a]
human fetal skin fibroblast (HFSF)	1250± 1.9
Human colon adenocarcinoma cell line (SW480)	625± 1.1
Human breast cancer cell lines (MCF)	312± 1.8
human fetal liver fibroblast (HFLP)	1250± 2.1

[a] IC50 values were expressed as the mean±S.D., determined from the results of MTT assay in triplicate experiments

CONCLUSIONS

Chemical characterization and antimicrobial screening studies on plant-based essential oils could lead to a discovery of new natural antimicrobials In the present work we demonstrated, for the first time, the potent cytotoxicity activity of *D. ammoniacum* aginst four cancer and normal cell line. In previous our study, The results of antimicrobial assay of the *D. ammoniacum* oil by disc

diffusion method and MIC values indicated that the oil exhibited moderate to high antimicrobial activity against seven Gram-positive and Gram-negative bacteria [10]. On the basis of these results the essential oil *D. ammoniacum* has antimicrobial activity and low cytotoxicity activity provide a scientific basis for the traditional use.

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