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Chemical composition and cytotoxic activity of the essential oil from the aerial parts of *Dorema aucheri*

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ABSTRACT

Introduction: Herbal products are beneficial compounds with many applications in human life. In this study the chemical composition and cytotoxic activity of the essential oil of the aerial parts of *Dorema aucheri* were assessed.

Methods: The essential oil was extracted by hydrodistillation after drying the aerial parts of *D. aucheri*, collected from the mountains around Yasuj city in the South-West of Iran. The oil composition was determined by GC/MS. To evaluate *in vitro* cytotoxic activity, the apoptotic effects of the essential oil were investigated against SW48 and SW1116 colorectal cancer cell lines by (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium) bromide (MTT) assay and flow cytometry.

Results: The essential oil yield was obtained 0.02% (W/W). Twenty-five compounds were identified in the oil, and the main constituents were caryophyllene (*E*) (31.29%), Phytol (14.92%), gurjunene (β-) (9.84%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (8.7%), and *n*-hexadecanoic acid (8.09%). The MTT assay showed that the IC_{50} values of the essential oil for SW48 and SW1116 cell lines were 1.4 and 1.2 mg/mL, respectively. The results of flow cytometry showed that the essential oil significantly increased the apoptosis in SW48 cell line compared with the vincristine ($P < 0.05$). It also increased the apoptosis in SW1116 cells compared with the vincristine, but this difference is not significant.

Conclusion: The essential oil of *D. aucheri* consisted of high amounts of caryophyllene and showed significant cytotoxic effects against SW48 and SW1116 cancerous cell lines.

Implication for health policy/practice/research/medical education:
The essential oil of *D. Aucheri* comprised of high amounts of caryophyllene and showed significant cytotoxic effects against SW48 and SW1116 cancerous cell lines. Hence, after more comprehensive studies, it might be used as a beneficial herbal source for developing anti-tumor drugs.

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Introduction

Dorema aucheri is a plant of Apiaceae family which grows at the end of the spring in southern provinces of Iran, especially in the provinces bordering the margins of the Zagros mountains, such as Kohgiluyeh and Boyer-Ahmad (1,2). The plant has medicinal properties and also is used by the local inhabitants for preparing food (1,3-5). It has already been proven that the aerial parts of the *D. aucheri* are rich in flavonoids (6). Flavonoids represent a large group of polyphenolic compounds that exhibit anti-oxidative effects (7,8). Although several reports have been conducted on the phytochemistry and

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bioactivity of hydroalcoholic extract of *D. aucheri,* the compounds and bioactivity effects of its essential oil have not been clearly determined. Several studies have reported hepatoprotective, anti-diabetic, anti-tumor, anti-oxidant, anti-hyperlipidemic, and anti-hypercholesterolemic effects of the hydroalcoholic extract of this plant (1,3-5,9). Moreover, in several pathophysiological states, it has been reported that *D. aucheri* extract has positive effects on thyroid hormones, antioxidant enzymes, the haematologic system and also serum levels of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (10). The analysis of essential oil extracted from the leaves of *D. aucheri* showed that it contained 36 (99.86%) compounds, and the major constituents included curzerene (18.7%), α-eudesmol (7.72%), Spathulenol (6.68%), isohibaene (6.16%), and gemberen (6.66%) (11). In several studies concerning the extracts of the aerial parts of *D. aucheri*, the presence of a large group of terpenoids, more specifically the sesquiterpene compounds, have been demonstrated. It has been indicated that the pharmacological features of *D. aucheri*, and its potential role in anti-inflammatory, and in the treatment of thyroid disorders and tumors can be due to its sesquiterpene compounds (12). In the present study, the constituents and bioactivity of the essential oil of *D. aucheri* were determined by GC-MS analysis. Also, the cytotoxic effects of these compounds were evaluated against two colorectal cancer cell lines (SW48 and SW1116).

Materials and Methods

Plant materials

The aerial parts of *D. aucheri* were collected from the mountains near the Yasuj city (25 km away from the city, at 30.4658640 N, 51.6783400 E, and the altitude of 2430 m) in Kohgiluyeh and Boyer-Ahmad province, Iran, in the spring of 2017. The plant was authenticated by a botanist (Dr. Azizollah Jafari, a botanist at Yasuj University, faculty of science). The aerial parts were dried in a dark place and then were powdered by an electric grinder. The voucher specimen of the authenticated plant (voucher no. 0496) was deposited at the herbarium of Medicinal Plants Research Center, Yasuj University of Medical Sciences.

Preparation of essential oil

The powder of *D. aucheri* (1200 g) was hydro-distilled in several runs for 4 hours using a Clevenger apparatus. The essential oil was collected, dried with anhydrous sodium sulfate, and kept in refrigerator until GC-MS analysis.

GC-MS analysis and identification of the oil components

The GC-MS analysis of the oil was conducted using a Hewlett-Packard 6890 instrument equipped with a HP-5M capillary column (phenyl methyl siloxane, 25 m × 0.25 mm id, Hewlett–Packard Part No. 190915.433, USA). The oven temperature was adjusted from 50°C (3 minutes) to 250°C at the speed of 3°C min^{-1} and finally continued for 10 minutes at 250°C. The injection temperature was 250°C. Helium was used as the transferor gas at a constant flow rate of 1.2 mL/min. The mass spectrometer (Hewlett-Packard 5973, USA) was activated in the electron ionization (EI) mode at 70 Ev and the mass range was 30-600 m/z. The identification of components was performed by comparing the relative retention times with those of a series of *n*-alkane standards (C10 to C30: ref. no. R-8769, Sigma) and linear interpolation based on computer matching with the Willey library (Willey-275) and spectra literature data (13).

Cell lines and culturing

Human colorectal cancer cell lines (SW48 and SW1116) were obtained from the Pasteur Institute of Iran. The cell lines were cultured in RPMI-1640 supplemented with 10% fetal calf serum, 1% glutamine, and 100 U/mL penicillin/streptomycin. The cells were cultured in a humidified atmosphere at 37°C and 5% CO_2.

Cell proliferation assay

The cellular proliferation was assessed using MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) assay. The cells (5×10^4) were seeded in each well of a microplate, containing 100 µL of the RPMI medium supplemented with 10% FBS. After a 24 hours incubation, the cells were attached to the bottom of each well, and treated with *D. aucheri* essential oil at the concentrations of 0.2 to 1.6 mg/mL for 24 hours. Then, 5 mg/mL MTT reagent was added to each well, and the plate was incubated at 37°C for 4 hours. As the positive control, the cells were treated with vincristine (Sobhan Oncology Co., Iran). Next, the supernatant was removed, and 100 µL DMSO was added to each well. Finally, the optical density of wells was determined at 490 nm using a microplate reader (Stat Fax3200, Awareness Technology, USA).

Apoptosis assay

The SW48 and SW1116 cells (1×10^6 cells per well) were seeded in six-well plates and then treated with either medium alone (negative control), *D. aucheri* essential oil (1.4 and 1.2 mg/mL), or vincristine (0.05 and 0.04 mg/mL) for 24 hours. The cells were resuspended in a cold binding buffer, then stained with annexin V-FITC reagent (5 µL) and propidium iodide (PI) (5 µL), and incubated in the dark at room temperature for 15 minutes. After adding 500 µL of the binding buffer, fluorescence was read using a fluorescence-activated cell sorter (FACS) (BD Biosciences, San Diego, CA, USA). Flow cytometry data was analyzed by FlowJo software. All the samples were assayed in triplicate.

Statistical analysis

For statistical analysis, the data of cytotoxic activity was compared between different groups by the analysis of

variance (ANOVA) followed by Tukey's post hoc test. The probability value of $P < 0.05$ was considered to denote a statistically significant difference.

Results

The yield of the essential oil extraction was 0.02% (W/W). GC-MS chromatogram of essential oil from the aerial parts of *Dorema aucheri* is shown in Figure 1. The results of GC-MS analysis (Table 1) showed that there were twenty-five (98.39%) known compounds in this essential oil. The main constituents included caryophyllene (*E*) (31.29%), phytol (14.92%), gurjunene (β-) (9.84%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (8.70%), and *n*-hexadecanoic acid (8.09%).

Cytotoxic activity

MTT assay was used to determine the IC_{50} of *D. aucheri* essential oil on SW48 and SW1116 cell lines. The cells were treated with 0.2 to 1.6 mg/mL concentrations of *D. aucheri* essential oil (Table 2). In parallel, vincristine was used as a positive control. The result showed that the essential oil significantly ($P < 0.05$) inhibited the cell growth of SW48 and SW1116 cell lines. As shown in Table 3, the IC_{50} values (The inhibitory concentrations that could reduce 50% of SW48 and SW1116 cells) were 1.4 and 1.2 mg/mL for essential oil and 0.05 and 0.04 mg/mL for vincristine, respectively. The results showed that the SW1116 cell line was more sensitive than SW48 to *D. aucheri* essential oil. Also, it was shown that the SW48 cell line was more resistant than SW1116 to vincristine.

Table 1. List of the components of the essential oil of *Dorema aucheri*

Compound	Essential oil%	Kovats indices (K.I)
Linalool	0.37	1084
Ylangene	0.49	1367
Caryophyllene *(E)*	31.29	1413
Aromadendrene	0.94	1420
Gurjunene (β-)	9.84	1426
Barbatene (β-)	0.69	1432
Humulene (α-)	1.30	1445
Acoradiene (α-)	0.34	1458
Curcumene (α-)	1.78	1468
Selinene (β-)	1.01	1477
Selinene (α-)	0.74	1487
Cuparene	3.28	1490
Curcumene (β-)	0.47	1500
Bazzanene (β)	0.64	1509
Nerolidol (*E*-)	0.57	1534
Longipinanol	0.99	1555
Caryophyllene oxide	2.55	1562
Globulol	0.37	1566
Tridecanol (*n*-)	2.82	1576
Unknown	0.94	1622
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	2.85	1822
Cyclopentadecanolide	0.55	1826
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	8.70	1836
Unknown	0.53	1877
Ethyl Linoleolate	2.80	1909
n-Hexadecanoic acid	8.09	1943
Phytol	14.92	2092

Apoptosis

To explore the mechanism by which *D. aucheri* essential oil might exert its anti-proliferative effects on SW48 and SW1116 cell lines, we assessed apoptosis using Annexin V and PI assay. The respective dot plots of this analysis have been shown in Figure 2. Based on the IC_{50} values, the cells were exposed to the essential oil for 24 hours to stimulate apoptosis. Flow cytometry results indicated that, 24 hours incubation with the essential oil significantly elevated apoptosis in SW48 cell line, compared with the vincristine ($P < 0.05$). It also increased the rate of apoptosis in SW1116 cells compared with the vincristine, but this difference was not significant (Figure 3). The apoptosis rates in SW48 and SW1116 cell lines treated with *D. aucheri* essential oil were 14.93% and 7.1%, while the cells treated with vincristine showed 4.5% and 6.43% apoptosis rates, respectively. Also, the essential oil treatment significantly elevated apoptosis in SW48 and SW1116 cell lines, compared with the negative control ($P < 0.05$). The comparison of apoptosis rates between SW48 and SW1116 cell lines showed that *D. aucheri* essential oil induced a significantly higher apoptosis rate in the SW48

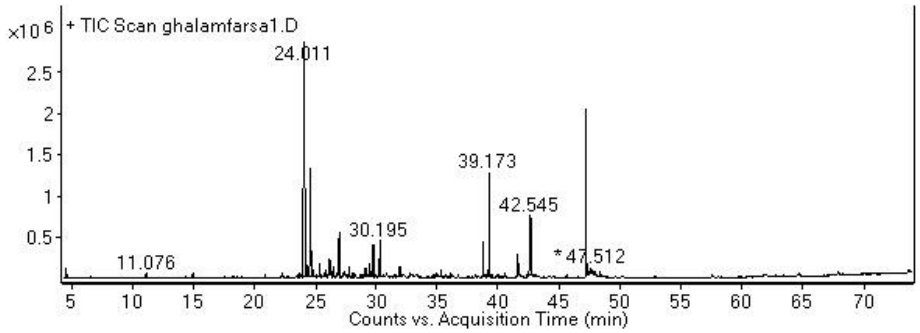

Figure 1. GC-MS chromatogram of essential oil from the aerial parts of *Dorema aucheri.*

Table 2. Effect of different concentrations of essential oil of *D. aucheri* aerial parts on viability of SW48 and SW1116 cells after 24 hours using MTT assay

Concentration (mg/mL)	Cell viability (%)	
	SW48	SW1116
0.2	92.42	91.23
0.4	90.54	89.71
0.6	88.38	85.42
0.8	75.36	67.31
1	68.39	60.03
1.2	57.43	50.44
1.4	49.27	47.12
1.6	43.64	42.81

SW48: Human colorectal cancer cell line, SW1116: Human colorectal cancer cell line.

Table 3. Cytotoxic activity of essential oil from the aerial parts of *D. aucheri*

Sample	MTT assay, IC50 (mg/mL)	
	SW48	SW1116
Essential oil	1.4 ± 0.05	1.2 ± 0.02
Vincristine	0.05 ± 0.001	0.04 ± 0.001

IC50: The half maximal inhibitory concentration, SW48: Human colorectal cancer cell line, SW1116: Human colorectal cancer cell line, MTT: microculture tetrazolium.
Results are expressed as means ± SD of three independent MTT assay performed in triplicate. Vincristine was tested as positive control.

cell line ($P < 0.05$).

Discussion

The current treatments for cancer, such as chemotherapy and radiotherapy, despite having cytotoxic effects against cancer cells, are associated with the side effects on normal proliferating cells. Therefore, it is required to develop alternative therapeutic approaches with the least possible complications (14-16). Among the potential sources for novel therapeutics, medicinal plants can be the most important options due to their anticancer components such as phenolics, glycosides, steroids, flavonoids, and terpenoids (17-19).

Although several studies have been carried out regarding the beneficial health effects of hydroalcoholic extract of the *D. aucheri* (3,4), there is inadequate knowledge about the composition and properties of the essential oil of this plant. In this study GC-MS analysis showed that terpenoids constituted 70.88% of the compounds identified in the investigated oil, among which sesquiterpenes (55.59%), diterpenes (14.29%), and monoterpenes (0.37%) were the predominant terpene compositions. Despite differences in the types of compounds, these compositions demonstrated similar biological functions compared to those of other plant species assessed in prior studies (7,8,20). Asnaashari et al who analyzed the composition of the essential oil of *D. glabrum* roots by GC/MS method showed that the oil was rich in sesquiterpenes and monoterpenes (21). In another study, the major constituents of the essential oil of *D. ammoniacum* collected from the Kellar mountain, were three hydrocarbon monoterpenes, five oxygenated monoterpenes, ten sesquiterpene hydrocarbons, and thirteen oxygenated sesquiterpenes (22). Akbarian et al in their study, conducted on five *D. aucheri* populations in different regions of Iran, showed that β-caryophyllene, thymol, β-gurjunene, carvacrol, and cuparene were the major components (23). In contrast, in the study of Delnavazi et al on *D. glabrum* plant, although the main components were non-terpene compounds (56%), terpenes were also widely found in the plant (24).

In our study, the analysis of essential oil of *D. aucheri*

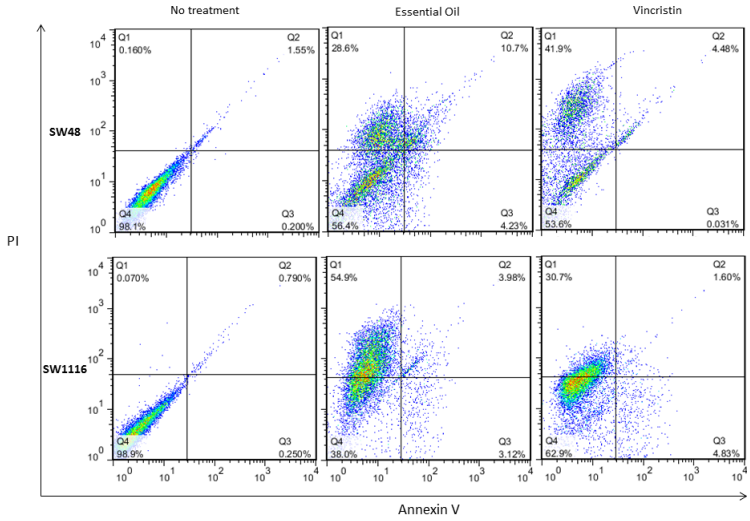

Figure 2. Flow cytometry results showing the percentage of apoptotic (early and late apoptosis), live, and necrotic cells following the treatment of SW48 and SW1116 cell lines with *D. aucheri* essential oil and vincristine. The treated cells with the essential oil and vincristine have shown significant increase ($P < 0.05$) in the proportion of cells entering necrosis stage. Also, compared to vincristine, the essential oil significantly elevated apoptosis in SW48 cell line. NT: no treatment, PI: Propidium iodide. Q1: necrosis, Q2: late apoptosis, Q3: early apoptosis, Q4: alive.

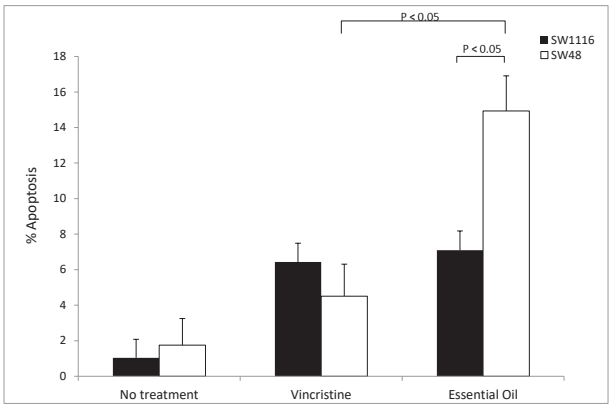

Figure 3. The apoptotic effects of the essential oil of *D. aucheri* on SW48 and SW1116 cell lines. The data represent mean ± SEM.

showed that α-eudesmol was absent, while this compound has been identified in other species of *Dorema*. In the analysis of the oil extracted from *D. aucheri* collected from the Hezar mountain, the main components were α-eudesmol (31.2%) and δ-cadinene (10.9%) (25). Also, the chemical composition of the essential oil from the stems and seeds of *Dorema ammoniacum* revealed a remarkable difference with our study (26). The reason for the discrepancy between our findings and previous ones can be due to the different environmental conditions leading to qualitative and quantitative variations in the compositions of oils (27).

Because terpenoids have been found to suppress the growth of a variety of cancer cells, the aim of the present study was to evaluate the biological effects of the essential oil of *D. aucheri* on SW48 and SW1116 colorectal cancer cell lines (28). The MTT assay was performed to evaluate *in vitro* cytotoxic activity of the essential oil against the cancer cell lines. The results showed that the essential oil of *D. aucheri* inhibited the growth of these cells as compared to vincristine. Our results also showed that the oil extract had greater toxic effects against the SW1116 cell line with the IC_{50} of 1.2 mg/mL compared to the value of 1.4 mg/mL obtained for the SW48 cell line.

We also evaluated the apoptotic effects of essential oil of *D. aucheri* against the mentioned cell lines using Annexin V and PI assay. The flow cytometry results indicated that 24 hours incubation with the extract significantly elevated apoptosis in SW48 cell line, compared to vincristine. The essential oils of *D. glabrum* and *D. ammoniacum* have been shown to effectively induce apoptosis in cancerous cell lines (29,30). Many studies have also demonstrated that the essential oil constituents of plants such as terpenoids present anti-cancer and pro-apoptotic effects and inhibit the differentiation, angiogenesis, invasion, and metastasis of tumor cells (28,31,32). In the present study, caryophyllene, a type of sesquiterpene, comprised the major compound (31.29%) of essential oil of *D. aucheri*. Several studies have shown the anti-cancer effects of this compound on osteosarcoma and breast tumors (33-35). Phytol, as a diterpene with anticancer effects (36), was the second major compound of *D. aucheri* essential oil with a concentration of 14.92%. This compound can be considered a possible candidate for a wide range of applications in pharmaceutical, food, and biotechnological industries (37).

Conclusion

The essential oil of *D. Aucheri* comprised of high amounts of caryophyllene and showed significant cytotoxic effects against SW48 and SW1116 cancerous cell lines. Hence, after more comprehensive studies, it can be used as a beneficial herbal source for developing anti-tumor drugs.

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Authors' contribution

SAH, GGH, FJN, AG, SH and BM contributed in designing the study, supervising , editing the manuscript and analyzing the data. GGH, FGH, SRY participated in the writing process. SAH, FGH, ZS and ML performed experiments.The final manuscript was read and approved by all authors.

Conflict of interests

The authors have no conflict of interests to declare.

Ethical considerations

This study was approved by the Ethical Committee of Yasuj University of Medical Sciences, Yasuj, Iran (ir.yums.rec.1395.213). Ethical issues (including text plagiarism, data fabrication and redundant publication) have been carefully observed by the authors.

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