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Essential Oil Analysis, Antioxidant and Antimicrobial Activity Evaluation of *Astrodaucus orientalis* (L.) Drude Grown in Iran

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Abstract

The genus *Astrodaucus Drude* (family: Apiaceae) is grown in Iran, Iraq, Syria. From temperate regions of Asia, it is raised in Turkey, and from Europe, it is rised in Ukraine. In this research, the methanol extract and essential oil constitution and in-vitro antioxidant and antimicrobial activity of the essential oil of *Astrodaucus orientalis* were evaluated. GC and GC/MS of the essential oil analysis of the overhead parts showed 73 components representing 99.25% of the oil. Trans-Anethole (57.84%), α-pinene (14.97%), sabinene (5.10%), and β-eudesmol (3.13%) were acquire as the significant components consisting 81.4% of the oil in the aerial parts. The essential oil analysis of the stems and roots showed 44 constituents representing 94.41% of the oil. β-eudesmol (17.20%), palmitic acid (11.40%), spathulenol (8.45%), and α-eudesmol (8.02%) were detected as the major components containing 45.07% of the root extract. The methanol extract was also subjected to screenings to evaluate its antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH), and β-carotene–linoleic acid tests. The methanol extract of leaves and inflorescences had considerable free radical scavenging activity in both DPPH (IC_{50} = 53.91 µg/ml), and β-carotene/linoleic acid (inhibition percentage: 68.08%) tests. However, this activity of the methanol extract in stems and roots was not considerable in DPPH (IC_{50} = 357.64 µg/ml), and β-carotene–linoleic acid (inhibition percentage: 43.07%). Total phenolic content (87.23 and 12.78 µg/mg) was also detected for the methanol extract of the aerial parts and the roots, respectively, as gallic acid equivalent in the Folin–Ciocalteu test. Also, its antimicrobial activity was screened, and good results were recorded for root extract against one of the microorganisms.

Keywords: *Astrodaucus orientalis*, Essential oil, Extract, GC/MS analysis, Antioxidant activity, Total phenolic, antimicrobial activity

Introduction

In recent decades, with the observation of the side effects of synthetic drugs, the return to medicinal plants that had been forgotten has received new attention to the extent that the 21st century can be called the century of the service and study of medicinal plants. Iran is a rich origin of other officinal plants. These grow wild in different parts of the country. Among 3000 species of medicinal plants known globally, 140 species are specific to Iran, and today traditional medicine in Iran has found many supporters (Judd et al., 1999). Umbrella plants grow mainly in temperate regions. Many species are confined to the northern highlands, and are also found in the tropics. The products of this family have medicinal and economic values, among which are the essential oils they contain (Margaris et al., 1982). This family, in terms of content and compounds, containing oils, essential oils, and internal secretions, can encompass more than 30 families related to the genus Umbelliferae. About 760 different combinations of essential oils have been isolated, and studied from these dark umbrella products (Mohamed and Abdu, 2004). Essential oils from the umbrella family are finely divided into phlyctenules placed between cells, were hormones act as regulators and catalysts in plant metabolism. Essential oils in the Umbelliferae family can also have antimicrobial activity versus *Staphylococcus aureus*, *Escherichia coli*, *Shigella*, and *Vibrochloria*, such as *Seseli libanotis* (Syed and Winlow, 1989; Özcan and Chalchat, 2006).

drug discovery is the ethnobotany data approach, in which the selection of a plant is based on the information on the use of the plant in traditional medicine. It is generally known that ethnobotany data provides an increased chance of finding active plants relative to the random approach (Lee, 1999; Montbriand, 2004). Thus, *A. orientalis* (L) Drude, a plant of the Umbelliferae family as a remedy for cancer-related diseases, was evaluated for its attributes. In Iran, the genus *Astrodaucus* is represented by two species; *A. persicus* (Boiss.) Drude, and *A. orientalis* (L.) Drude, which grows ferine in different regions of Iran and nearby countries, such as central and southern Russia, northern and north-western Caucasus, the western desert of Syria, inner Anatolia and central Asia. In Iran, *A. orientalis* is mainly distributed in Azerbaijan, Ghazvin, Mazandaran, and Khorasan provinces. The chemical composition of the essential oils of the leaves and the seeds of *A. orientalis* was investigated, but this plant's cytotoxicity and antimicrobial activities have not been reported previously (Mirza et al., 2003). It was also shown that the extract of the root and the overhead parts of *A. Persicus*, another species of the genus in Iran, exhibited a potent antiproliferative effect on the T47D cell line, human breast cancer cells (phytochemical properties, s.m. Razavi). Thus, *Astrodaucus persicus* (Boiss.) Drude, a medicinal plant of the Umbelliferae family (Apiaceae) used to remedy cancer-related diseases, has been evaluated for its properties.

This study was aimed to identify the essential oil compositions of the aerial parts and the roots of the plant *A. orientalis*, and investigate the antioxidant and antimicrobial properties of the essential oil and the methanolic extract of the plant. The phenol content of the total methanolic extract is also determined in this work.

Materials and Methods

Materials

Plant Materials

The whole plant (leaves, stems and roots) of *A. orientalis* were gathered during the flowering time in May 2014 from Kashan (Isfahan province in Iran) at a height of ca. 2900 m. The plant voucher specimen was deposited in the herbarium of the Research Institute of Rangelands and Forests, Kashan, Iran.

Chemical Material

β-carotene, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, linoleic acid, 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene, BHT), and gallic acid were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Analytic class methanol, ethanol, dimethylsulfoxide (DMSO), chloroform HPLC grade, working standard of folin-ciocalteu phenol reagent, anhydrous sodium sulfate, sodium carbonate, Tween 40, linalool, dodecane, bornyl acetate, bisabolol, and all culture media were acquired from Merck (Darmstadt, Germany). Water for injection was used for the assays.

Preparation of the Extracts

Isolation of the Essential Oil

The essential oil was extracted by crushing the seeds in a blender until they became a powder. Then the essential oil was extracted by the Hydrodistilation method using Clevenger for 3.5 hours. The essential oil was dried without sodium sulfate and water. After elutriating and drying over anhydrous sodium sulphate, yellow-colored oils were recovered and stored at low temperature (4°C) under a nitrogen atmosphere in Aluminium vials, and were used for analyses within a few days.

Chromatographic Analysis

Gas Chromatographic Analysis

Essential oil samples attained from the aerial parts of *A. orientalis* were analyzed by using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an FID detector using a HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Restek, Bellafonte, PA). Oven temperatures were programmed as follows: 50°C (2 min), 50–130°C (5°C/min), 130°C (2 min), and 130–200°C (3°C/min). Detector and Injector were set at 220°C and 290°C, respectively. Ultra-high purity nitrogen (flow rate: 50 ml/min), helium (flow rate: 1 ml/min), and hydrogen (flow rate: 40 ml/min) were used as carrier, fuel, and makeup gases, respectively. Compressed air was used for combustion (flow rate: 450 ml/min). 1.0 µl of samples was injected manually in the splitless mode. The percentage of each compound relative to the peak area of the entire spectrum (100%) were used for obtaining its quantitative data. The injection was repeated three times, and the peak area percent as means ± SD of triplicates was reported. The commercially available co-injection components available from essential oils were also used, and lead to the affluence of the mentioned peaks in the spectrum and further validity of their accommodation.

GC/MS Analysis

GC/MS Oil Analysis was fulfilled by the Gas Chromatography HP-6890 Agilent (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (ionization energy: 70 eV), operating under the same conditions as explained above, using a HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Restek, Bellafonte, PA). The retention index is calculated for all components using the N-Alkane homolog series injected in the same condition as sample one. comparison of essential oil components is based on the retention index (RI) relative to N-Alkane, and computers matching with the Library of Wiley275.L and Wiley7N.L, and comparisons of mass spectral fragmentation patterns with data published in the literature (Adams, 2007).

Antioxidant Activity

DPPH Radical Assay

Because of this mean, solutions with different concentrations (2 to 5 × 10-10 mg/ml) of the extract, and the synthetic antioxidant Butylated Hydroxy (BHT Toluene) in methanol were prepared. 1 ml of DPPH methanolic solution (at a concentration of 1 mM equivalent to 0.394 mg) was added to 3 ml of the extract, and the resulting mixture was stirred vigorously. The test tubes were placed in a dark place for 30 minutes. After this period, the absorbance was read at 517 nm. It should be attended that in the control sample, the extract was replaced with 3 ml of methanol. Finally, the percentage of inhibition of DPPH radicals by the extract was calculated by the subsequent formula:

$$\text{Inhibition \%} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

A_{sample} is the absorbance rate of the test compound, and A_{blank} is the absorbance rate of the control reaction.

β-Carotene/Linoleic Acid Bleaching Assay

According to the method (Juntachote and Berghofer, 2004), a spectrophotometer with a wavelength of 490 nm was used. For the experiment, a base solution of beta-carotene-linoleic acid was prepared as follows: 0.5 mg of beta-carotene was dissolved in 1 ml of chloroform, then 25 µl of linoleic acid, 200 mg of tween 40 were also added and blended completely. The chloroform was then separated by vacuum evaporation, and 100

ml of oxygen-saturated distilled water (30 min at 100 ml/min) was added with strong shaking. 5.2 ml of the above solution was transferred to the test cell, and then 350 µl of the extract (concentration two g/l in methanol) was added to the test tube as well. Test tubes were incubated in a water bath at 50°C for 2 hours, and negative control (blank) containing the same methanol volume instead of the extract. The absorbance value was measured at 470 nm in the invisible and visible spectrometer (UV-Vis) (Cintra 6, GBC, Dandenong, Australia). The level of antioxidant activity was estimated by comparing the light absorption of the samples with zero time and the stability of beta-carotene yellow colour as a percentage.

Assay for Total Phenolic

Total phenolic content was estimated using folin-Ciocalteu's reagent. To 0.1 ml of each extract (1000 µg) 2.5 ml of 0.2 normal folin reactant was added; after 5 minutes, 2 ml of 75 g/l sodium carbonate solution was added. After 2 hours, the adsorption of the mixture at 760 nm was read by the spectrophotometer in presence of Blanc; gallic acid was used as the standard to represent the calibration curve. Total phenolic content was reported based on the equivalent of mg of gallic acid per gram of the extract. The tests were repeated three times, and their average was reported (Slinkard and Singleton, 1977).

Antimicrobial Activity

Microbial Strains

The microbial strain used in this investigation is performed by the Iranian research organization of science and technology (IROST): *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 10536), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737), *Klebsiella pneumoniae* (ATCC 10031), *Staphylococcus epidermidis* (ATCC 12228), *Shigella dysenteriae* (PTCC 1188), *Salmonella paratyphi*-A serotype (ATCC 5702), *Proteus vulgaris* (PTCC 1182), *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404), and *Aspergillus brasiliensis* (PTCC 5011).

Disc Diffusion Assay

Agar disk-diffusion measurement developed in 1940 (Heatley, 1944) is the official method used in many clinical microbiology laboratories for conventional antimicrobial susceptivity testing.

In this method, nutrient agar media plates were seeded with 18-24 hour cultures of microbial inoculums (a standardized inoculum of 1-2 × 107 CFU ml-1 0.5 McFarland Standard). Whatman No. 1 filter paper discs (6 mm in diameter) were assigned with the help of sterile forceps on the media, and then plant extracts in concentrations of 1, 2, and 3 mg disc-1 in 6, 12, and 18 µl volume were applied on the discs. Antibiotics (6 µl disc-1) as positive controls and dimethyl sulfoxide (DMSO) (6 µl disc-1) as negative control were also applied on the discs. Inoculated plates were then incubated at 37°C for 18-24h. The next day, zones of inhibition were recorded in mm throughout the discs in each plate. Azithromycin was the positive control for Gram-positive bacteria (50 µg 6 µl-1); for Gram-negative bacteria, it was ciprofloxacin (30 µg, 6 µl-1), and for Candida albicans, it was clotrimazole (50 µg, 6 µl-1).

The same method was followed thrice for each microorganism to get the best possible results and clear any doubt.

Micro-well Dilution Assay

The broth microdilution method was used to restrict the minimum inhibitory concentration (MIC) of the *A. orientalis* essential oil against multi-resistant strains separated from wounds. The inocula of the bacterial strains were prepared from overnight broth cultures, and suspensions were adjusted to 0.5 McFarland

standard turbidity (corresponding to 107 -108 CFU/ml for bacteria, depending on genera (consensus standard by the NCCLS M38 [ISBN 1-56238-480-8]). The examined oil was dissolved in 1% dimethylsulphoxide (DMSO), and then diluted to the highest concentration (500 µl/ml). A serial doubling dilution of the oil was provided in a 96-well microtiter plate over the range of 0.078 to 5 mg/ml in 10 ml in an inoculated nutrient broth. The plate was incubated for 24 h at 37°C. MIC was defined as the lowest concentration of essential oil, which inhibited the obvious growth of microorganisms. The microbial growth was determined by absorbance at 620 nm using the universal microplate reader. All determinations were performed in duplicate, and two growth controls consisting of nutrient broth with 1% DMSO (v/v) were combined.

Results and Discussion

Chemical Composition of the Essential Oil

Aerial parts and roots of the plant were subjected to hydro-distillation using a Clevenger-type apparatus, and the yellow-colored essential oils were obtained (yield 0.055 ± 0.04 ml, 0.036% v/w; 0.0085 ± 0.07 ml, 0.085% v/w based on the weight of the dried plant material of the aerial parts and the roots respectively). GC and GC/MS analysis resulted in identifying 14 compounds representing 98.74% of the oils for the aerial parts (Table 1) and 15 compounds representing 90.62% of the oils for the roots (Table 2). The plant's essential oil consisted principally of monoterpenes with non-oxygenated besides oxygenated alkene structures. Trans-Anethole (57.84%), α-pinene (14.97%), sabinene (5.10%), and β-eudesmol (3.13%) were the major components containing 81.04% of the oil of the aerial parts:

Table1. *Chemical composition of the aerial parts of essential oil of A. orientalis*

No	Compound[a]	Composition (%)	RI[b]	RI[c]
1	α-Pinene	14.97	932	939
2	Camphene	0.57	939	936
3	Sabinene	5.10	967	961
4	(-)-β-Pinene	1.59	970	974
5	p-Cymene	2.27	10021	1019
6	γ-Terpinene	0.85	1054	1049
7	(-)-Bornyl acetate	2.72	1280	1282
8	trans-Anethole	57.84	1313	1301
9	α-Copaene	1.69	1376	1376
10	β-Cubebene	1.95	1390	1390

11	α-Curcumene	1.90	1482	1481
12	(+)-Spathulenol	2.91	1588	1581
13	β-Eudesmol	3.13	1666	1662
14	α-Eudesmol	1.25	1668	1669
Total		98.74		

[a] Compounds are listed in order of elution from HP-5MS column.

[b] Relative retention indices to C8-C24 n-alkanes on HP-5MS column.

[c] the Literature retention indices.

Furthermore, β-eudesmol (17.20%), palmitic acid (11.40%), (+)-spathulenol (8.45%), α-eudesmol (8.02%), Bicyclosesquiphellandrene (6.86%), and bornyl acetate (6.74%) were the major components containing 58.67% of the oil of roots:

Table2. *Chemical composition of the roots of essential oil of A. orientalis*

No	**Compound[a]**	**Composition (%)**	**RI[b]**	**RI[c]**
1	Bornyl acetate	6.74	1280	1285
2	trans-Anethole	3.59	1283	1283
3	α-Longipinene	2.95	1344	1342
4	α-Copaene	3.76	1370	1369
5	β-Cubebene	2.77	1385	1386
6	β-Cadinene	2.63	1520	1519
7	(+)-Spathulenol	8.45	1580	1581
8	Bicyclosesquiphellandrene	6.86	1649	1521
9	β-Eudesmol	17.20	1664	1662
10	α-Eudesmol	8. 02	1666	1669
11	6,10,14-trimethyl-2-Pentadecanone	4.95	1852	1846
12	Phytol	3.24	1869	2111

13	Palmitic acid	11.40	2003	2003
14	β-Ocimene	3.70	2159	1044
15	Methyl isopimarate	4.36	2295	2298
Total		90.62		

[a] Compounds are listed in order of elution from HP-5MS column.

[b] Relative retention indices to C8-C24 n-alkanes on HP-5MS column.

[c] the Literature retention indices.

The papers on the chemical composition of the essential oils of the plants of *Astrodaucus* genus are scarce in the literature. Bornyl acetate, β-sesquiphellandrene, and Exo-fenchyl acetate for the root essential oil, α-pinene and Exo-fenchyl acetate for the stem/leaves, and β-pinene, α-pinene, and α-thujene for the flowers/fruits oils were also found in the essential oil of *A. orientalis* as major components (Bazargani et al., 2006), sabinene, α-pinene, and fenchyl acetate for essential oil of fruit and α-copanene, β-pinene, and sabinene as the major components of essential oil of umbels and anisole; bornyl acetate and geranyl tiglate were also observed in considerable amounts in the oil of *A. orientalis* collected from the north-west of Iran (Nazemiyeh et al., 2009). Finally, this investigation, compared to other same works, reveals well accordance to other samples in different regions of Iran and the world.

Antioxidant Activity

Antioxidant activity is a complex process that usually occurs through several mechanisms. Because of its complexity, the estimate of antioxidant activity for pure compounds or extracts must be done with more than one research method (Aruoma, 2003). In this work, two established antioxidant essays, namely DPPH and β-carotene/linoleic acid tests, were carried out together with Folin–Ciocalteu's essay, which estimates the total phenolic content of the plant extract.

During the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical test, the capacity of the samples to give hydrogen atoms and electrons to this blue/purple stable radical, and converting it to a yellow diphenylpicrylhydrazine molecule was estimated (Tepe et al., 2005). This reaction is used to estimate the extracts or the pure molecules (like BHT) to scavenge free radicals. The outcomes of this test are displayed in Table 3. Compared to the synthetic standard antioxidant BHT (IC_{50} = 16.13 µg/ml), the extract of the aerial parts of the plant showed rather good radical-scavenging activities because of the free radical scavenging activity of the methanol extract (IC_{50} = 53.91 µg/ml). The root-extracts exhibited weak radical-scavenging activities (IC_{50} = 357.64 µg/ml). Due to the major contribution of phenolic compounds in antioxidant activity, the significant antioxidant activity of the aerial parts of the extract may be a consequence of its high phenolic compounds content, which was reflected in its Folin–Ciocalteu's test result (87.23 µg/mg as gallic acid equivalent). Another contributing factor might be the major enrichment of phenolic compounds in the antioxidant activity. The weak antioxidant activity of the root extracts may be the outcome of its low phenolic compounds content which was reflected in its Folin–Ciocalteu's test result (12.78 µg/mg as gallic acid equivalent). Potential capacities of the antioxidants to prevent lipid peroxidation by reacting with radical peroxyl chains faster than the radical reaction with protein or with the side connections of fatty acids is usually evaluated by the β-carotene/linoleic acid experiment (Tepe et al., 2005; Sacchetti et al., 2005 and Trouillas et al., 2003).

The methanol extract of the plant revealed an inhibition percentage (68.08% and 63.28%) similar to that of synthetic standard BHT (95.6%, Table 3) for the aerial parts and the roots, respectively.

Table 3. *Antioxidant activity of the aerial parts and roots of methanol extract of A. orientalis and BHT in DPPH free radical scavenging activity and β-carotene/linoleic acid bleaching assay techniques*

Sample	**DPPH**	***β*-carotene/linoleic acid**
	IC_{50} (µg/ml)	**Inhibition (%)**
Aerial part	53.91 ± 1.49	68.08 ± 0.018
Root	357.64 ± 0.97	63.28 ± 0.04
BHT	16.13 ± 0.42	96.24 ± 0.025
Blank	NA	2.57 ± 0.015

[a] Fewer than 9% inhibition for the essential oil concentration of higher to 10 mg/ml, ND (Not determined), NA (Not applicable).

Antimicrobial Activity

The antimicrobial activity of *A. orientalis* methanol extracts against a panel of 12 microorganisms was tested, and their strengths were estimated both qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters, and MIC values. The results are given in Table 4. The methanol extract of the roots showed weak antimicrobial activities against *A. orientalis* in disc diffusion, and micro-well dilution tests. The methanol extract of the aerial parts showed no appreciable antimicrobial activity. The roots of *A. orientalis* showed a more effective and wider spectrum of antimicrobial activities than the methanol extract of the aerial parts.

Table 4. *Antimicrobial activity of the aerial parts and roots of methanol extract of A. orientalis*

	Aerial parts		Roots		Antibiotics					
					Rifampin		Gentamicin		Nystatin	
Test microorganisms	DD[a]	MIC[b]	DD	MIC	DD	MIC	DD	MIC	DD	MIC
P. aeruginosa ATCC 27853	-	-	-	-	-	-	8	0.50	NA	NA
B. subtilis ATCC 6633	-	-	-	-	13	0.125	21	0.50	NA	NA
E. coli ATCC 10536	-	-	-	-	11	0.50	21	0.50	NA	NA
S. aureus ATCC 29737	-	-	-	-	10	0.250	21	0.50	NA	NA
K. pneumoniae ATCC 10031	-	-	-	-	7	0.250	22	0.25	NA	NA
S. epidermidis ATCC 12228	-	-	9	2.50	40	0.250	35	0.50	NA	NA
S. dysenteriae PTCC 1188	-	-	-	-	8	0.250	18	0.50	NA	NA
P. vulgaris PTCC 1182	-	-	-	-	10	0.125	23	0.50	NA	NA
S. paratyphi-A serotype ATCC 5702	-	-	-	-	-	-	21	0.50	NA	NA
C. albicans ATCC 10231	-	-	-	-	NA	NA	NA	NA	33	0.125
A. niger ATCC 16404	-	-	-	-	NA	NA	NA	NA	27	31.2
A. brasiliensis PTCC 5011	-	-	-	-	NA	NA	NA	NA	30	31.2

A dash (-) shows no antimicrobial activity.

[a] Inhibition zone in diameter (mm) around the impregnated discs.

[b] Minimal Inhibition concentrations (as mg/ml).

[c] Plant samples were inactive against mold in the disc diffusion test so that the MIC agar dilution test for nystatin was not carried out. NA (Not applicable).

Conclusion

Considering the potential antioxidant activity and rich amounts of phenolic compounds in *A. orientalis*, this plant extract can be used in the pharmaceutical and food industry instead of synthetic antioxidants and other chemical preservatives to delay lipid peroxidation, and prevent the growth of pathogens.

Due to the adverse effects of chemical compounds on human health and the resistance of bacterial strains to antibiotics, more studies on the application of this extract in biological systems are suggested. In this respect, our study can be considered as the first report on the in-vitro antioxidant and antimicrobial activity

of the methanol extract of different parts of the edible plant *A. orientalis*. The plant extracts appreciable antioxidant activity, especially in the aerial parts, in the β-carotene/linoleic acid and DPPH tests encourage more extensive investigations in this respect.

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