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Essential oils chemical composition, antioxidant activities and total phenols of *Astrodaucus persicus*

Saeid Goodarzi [1], Abbas Hadjiakhoondi [1], Narguess Yassa [1], Mahnaz Khanavi [1], Zahra Tofighi [1*]

[1] Department of Pharmacognosy, Faculty of Pharmacy and Medicinal Plant Research Center, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Objective(s): *Astrodaucus persicus*, Apiaceae, is used as vegetable or food additive in some parts of Iran. The essential oils of different parts of *Astrodaucus persicus* from Kordestan province were analyzed for the first time and compared with other regions. In this study, antioxidant activities and total phenols determination of aerial parts essential oils and root fractions of *A. persicus* were investigated.

Materials and Methods: The essential oils were obtained by hydro-distillation from flowers/fruits, leaves/stems, ripe fruits and roots of plant and analyzed by GC-MS. Crude root extract was fractionated with hexane, chloroform, ethyl acetate and methanol. Antioxidant activities by DPPH and FRAP methods and total phenols by Folin-ciocalteu assay were measured.

Results: The abundant compounds of flowers/fruits blue essential oil were α-thujene, β-pinene and α-pinene. The predominant components of blue leaves/stems essential oil were α-thujene, α-pinene and α-fenchene. The major volatiles of ripe fruits blue essential oil were β-pinene, α-thujene and α-pinene. The chief compounds of root yellow essential oil were trans-caryophyllene, bicycogermacrene and germacrene-D. Total root extract and ethyl acetate fraction showed potent antioxidant activities and high amount of total phenols in comparison to other samples. Among volatile oils, the flowers/fruits essential oil showed potent reducing capacity.

Conclusion: The major compounds of aerial parts essential oils were hydrocarbon monoterpenes while the chief percentage of roots essential oil constituents were hydrocarbon sesquiterpenes. α-Eudesmol and β-eudesmol were identified as responsible for creation of blue color in aerial parts essential oils. *A. persicus* was known as a potent antioxidant among Apiaceae.

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Introduction

Astrodaucus is a genus of Apiaceae which is represented by two species, *Astrodaucus persicus* Boiss. Drude and *Astrodaucus orientalis* L. Drude. This genus grows wild in different regions of Iran and nearby countries such as Russia, Syria, inner Anatolia, Trans-Caspia and Central Asia (1). *Astrodaucus* is traditionally used as salad, vegetable or food additive in some parts of Iran and Turkey (2). Determination of the nutrition contents of *A. orientalis* showed the high amount of iron (7.12 mg/100 g), manganese (0.90 mg/100 g) and copper (0.47 mg/100 g) (3).

Chemical composition of *A. persicus* essential oils from Qazvin and Tehran and components of *A. orientalis* volatile oils from Zanjan and Tehran were investigated in previous studies (1, 4-7).

There were a few biological investigations on *Astrodaucus* genus especially *A. persicus*. The study on cytotoxicity of *A. persicus* aerial and root extracts (especially root extract) showed strong anti-proliferative effects on T47D breast carcinoma cell by mechanisms such as apoptosis in comparison to negative control and doxorubicin (8, 9). The aerial and root extracts of *A. orientalis* demonstrated potent anti-proliferative effects on T47D cells by decrease in p53 and Bcl-2 protein expression which are believed to play a crucial role in tumorigenesis and cell death (10). In another study, methanol extract from roots of *A. orientalis* exhibited cytotoxic properties against Mc-Coy cell line with IC_{50} value of 349 µg/ml. Dichloromethane extract from roots of *A. orientalis* has significantly reduced shoot and root growth of seedlings in lettuce assay and so found to have phytotoxic ability (11).

The aqueous extract of flowering shoots of *Astrodaucus* sp. showed antibacterial activities against *Xanthomonas arboricola* pv. *juglandis,* which is caused the most destructive bacterial disease of the genus *Juglans* worldwide (12).

In this study, *A. persicus* was collected from Kordestan Province of Iran and the chemical composition of the blue essential oils of different aerial parts including flowers/fruits, leaves/stems and ripe

*Corresponding author: Zahra Tofighi. Department of Pharmacognosy, Faculty of Pharmacy and Medicinal Plant Research Center, Tehran University of Medical Sciences, Tehran, Iran. Tel: +98-2164121220; Fax: +98-2189771428; email: ztofighi@tums.ac.ir

fruits and yellow essential oil of roots were investigated and compared with each other and those of other regions. The total phenols and antioxidant activity of aerial parts essential oils and various fractions of root extract were determined by DPPH and FRAP methods and compared with positive controls for the first time. Investigation of antioxidant effects and total phenolic content is interesting because by comparison of these two markers with reported cytotoxicity of *A. persicus*, the correlation may be found.

Materials and Methods

Chemicals

Vitamin E 97% (Sigma-Aldrich Chemie GmbH, Germany); 2, 2-diphenyl 1-picrylhydrazyl (DPPH; Fluka, Switzerland); Butylatedhydroxylanizole (BHA), Sodium acetate, 2,4,6-tripyridyl-s-triazine (TPTZ), $FeCl_3.6H_2O$, $FeSO_4.7H_2O$, Na_2HPO_4, NaH_2PO_4, Folin-Ciocalteu phenol reagent, glacial acetic acid, hydrochloric acid, ethanol and methanol (Merck, Germany) were purchased.

Plant materials

A. persicus (Boiss.) Drude was collected from around Irankhah village, Saghez, Kordestan Provinces, Iran. The flowers/fruits and leaves/stems were gathered at flowering stage in June and ripe fruits and roots were prepared at fruiting stage in September 2010. The different parts were dried and powdered separately. Plant was identified by Mr Y Ajani and deposited in Herbarium of Institute of Medicinal Plants, ACECR, Karaj, Iran (No. 2844 MPIH).

Extraction and fractionation

The 1190 g of *A. persicus* roots was macerated with 80 % methanol (4 L) every 24 hr at room temperature until the solvent gained color and the extract was concentrated (42.5 g crude extract). Crude extract was fractionated with hexane (HE 15.63 g), chloroform (CL 7.44 g), ethyl acetate (EA 2.54 g) and methanol (ME 15.25 g).

Isolation of the essential oils

The air-dried flowers/fruits, leaves/stems, ripe fruits and roots of *A. persicus* were separately subjected to hydro-distillation for 4 hr using a clevenger type apparatus. The oils were collected separately, dried on anhydrous sodium sulfate and kept in refrigerator for GC and GC/MS analysis.

Gas Chromatography

The essential oils were analyzed using a Hewlett Packard 6890 gas chromatograph equipped with a HP-5MS column (5% phenylmethylpolysiloxane) (30 m × 0.25 mm, film thickness 0.25 µm). The thermal program was 40-250 ˚C at a rate of 3 ˚C/min; The Injector and detector (FID) temperatures were 250 and 230 ˚C respectively and split flow was adjusted at 50 ml/min. Helium (99.999 %) was used as carrier gas at a flow rate of 1 ml/min. The percentage compositions of the identified compounds were computed from the GC peak areas.

GC/MS analysis

The oils were analyzed by GC/MS using a Hewlett Packard 5973 mass selective detector connected to a HP 6890 gas chromatograph. The separation was achieved at the same gas chromatographic conditions. MS were taken at ionization potential of 70 eV. Identification of compounds was based on comparison of Kovats indices (KI) and fragmentation patterns of mass spectral data in comparison with standard compounds in Wiley library or published data in the literature (13, 14).

Antioxidant activity

DPPH –free radical scavenging activity

For investigation of radical scavenging activity of fractions, the DPPH method was used (15). One ml of different concentrations of each essential oil (20, 10, 5, 2.5 µl/ml) and fraction (25, 50, 100 and 250 µg/ml) were added to 2 ml of DPPH solution (4×10^{-5} g/ml MeOH). Methanol was added to negative controls which were containing maximum concentration of samples up to 3 ml; 2 ml of DPPH solution was added to blank (1 ml methanol). Vitamin E (40 µg/ml) and BHA (100 µg/ml) were used as positive controls. The absorbance was measured 30 min after at 517 nm and the radical scavenging activity was calculated as follow:

Inhibition % =100- [(Sample absorption-control absorption)/Blank absorption] ×100

All tests were carried out in triple replicate and IC_{50} were calculated.

FRAP - ferric reducing antioxidant power assay

The total antioxidant capacity of different essential oils and fractions of *A. persicus* were determined by measurement of their abilities to reduce ferric tripyridyltriazine (Fe(III)-TPTZ) complex to its ferrous colored form (Fe(II)-TPTZ) at low pH. (Fe(II)-TPTZ) has an intensive blue color and can be monitored with spectrophotometer (16). One and half ml of FRAP reagent [2.5 ml of 10 mM TPTZ solution in 40 mM HCl, 2.5 ml of 20 mM $FeCl_3$ and 25 ml of 0.3 M acetate buffer, pH 3.6] was added to 50 µl of each sample (100 µg/ml). After incubation at 37 ˚C for 10 min, the absorbance was measured at 593 nm. FRAP reagent used as blank and the experiment was performed in triplicate. Different concentration of aqueous solution of $FeSO_4.7H_2O$ (in a range of 125-1000 µmol/l) was used for calibration curve. The relative antioxidant activities of samples were reported as mmole Fe^{2+}/100 g of fractions.

Folin ciocalteu –total phenol assay

Total phenol content of all samples was determined by Folin Ciocalteau method (17). It involves the oxidation of phenols in alkaline solution by the yellow molybdotungstophosphoric heteropolyanion reagent and colorimetric measurement of the resultant molybdotungstophophate blue. These blue pigments have a maximum absorption depending on the composition of phenol mixtures besides the pH of solutions, usually obtained by adding sodium carbonate or sodium bicarbonate (18). The methanol solution of prepared dilution of each samples and gallic acid as standard phenol compound (0.2 ml) were mixed with Folin-Ciocalteu reagent (2 ml, 1:10 diluted with distilled water) and after 5 min, saturated $NaHCO_3$ solution (1.5 ml, 60 g/l distilled water) was added. After 90 min incubation at room temperature, the absorption of the solutions was measured using spectrophotometer at 725 nm. The standard curve was prepared using 0, 25, 50 and 100 mg/ml solutions of gallic acid (GA) in methanol and total phenol compounds were expressed as gallic acid equivalents (GAE; mg of gallic acid per g of samples). All tests and analyses were carried out in triplicate.

Results

GC/MS analysis of essential oils

Volatile compounds of different parts of *A. persicus* from Kordestan province were investigated and demonstrated in Table 1.

The aerial parts essential oil samples were observed as blue color liquid and were obtained in yield of 0.6-0.9% (v/w) while the roots essential oil was seen as yellow color liquid in yield of 0.1% (v/w). The flowers/fruits essential oil contained monoterpenes (97.3%) and sesquiterpenes (1.4%), which was similar to leaves/stems essential oil included monoterpenes (96.5%) and sesquiterpenes (2.1%) and ripe fruits essential oil contained monoterpenes (95.9%) and sesquiterpenes (1.1%). The roots essential oil included monoterpenes (5.2%) and sesquiterpenes (90.7%). Investigation of essential oil of ripe fruits and roots showed the existence of nonterpenes (1.4 and 4.1%, respectively) which was not observed in two other aerial parts samples. It was interesting that the amount of monoterpenes was more than sesquiterpenes in aerial parts and vice versa the sesquiterpene content was abundant in roots of *A. persicus*. The major compounds of three aerial parts essential oils belonged to hydrocarbon monoterpenes which was calculated as 91.3%, 91.3% and 93.6% for flowers/fruits, leaves/stems and ripe fruits, respectively while hydrocarbon sesquiterpenes were abundant in roots essential oil (82.9%).

Antioxidant and total phenols determination

Antioxidant activities and total phenols of aerial parts essential oils and root different fractions of *A. persicus* in comparison with vitamin E and BHA (butylatedhydroxyanisole) as natural and synthetic antioxidants were reported in Table 2.

Discussion

GC/MS analysis of essential oils

The abundant compounds of flowers/fruits essential oil were α-thujene (43.8%), β-pinene (21.3%) and α-pinene (20.9%). The predominant components of leaves/stems essential oil were α-thujene (48.0%), α-pinene (27.7%) and α-fenchene (9.2%). The major volatiles of ripe fruits essential oil were β-pinene (56.9%), α-thujene (17.6%) and α-pinene (14.3%). Comparison of essential oils of unripe and ripe fruits showed that the amount of β-pinene was increased with maturation in ripe fruits while α-thujene and α-pinene contents were decreased. The similar major volatile components of three aerial parts essential oil samples were α-thujene, α-pinene, camphene, *p*-cymene, γ-terpinene, α-fenchyl acetate, bornyl acetate, γ-cadinene, β-eudesmol and α-eudesmol. The chief compounds of root essential oil were trans-caryophyllene (33.5%), bicycogermacrene (27.3%) and germacrene-D (11.6%). α-Pinene, γ-terpinene and bornyl acetate were common in aerial parts and roots essential oils.

α-Thujene, camphene, p-cymene, α-fenchyl acetate, γ-cadinene, β-eudesmol and α-eudesmol were compounds which existed in three aerial parts essential oils but there were not seen in roots volatile oil.

The previous investigation on chemical composition of *A. persicus* essential oils from Taleqan (Qazvin province) of Iran represented bornyl acetate, β-sesquiphellandrene and exo-fenchyl acetate as major compounds of yellow essential oil of root, α-pinene and exo-fenchyl acetate as abundant components of green essential oil of stem/leaves and β-pinene, α-pinene and α-thujene as main principles of bluish-green essential oil of flowers/fruits (1). Another research demonstrated the major compounds of yellow essential oil of *A. persicus* aerial part collected from northeast of Tehran were decanal, dodecanal and dodecanol (4). The abundant components of pale yellow essential oil of *A. persicus* seeds, cultivated in Northeast of Tehran were geranyl acetate, α-pinene and sabinen (5). Mirza *et al* examined the essential oils of *A. orientalis* leaves and seeds from Alamut (Zanjan province) in Iran. The sample oils were blue in color. The major components of the leaves oil were fenchyl acetate and α-pinene but the major constituents of the seeds oil were myrcene and β-pinene (6). The dominant components of aerial parts essential oil of *A. orientalis* from Fasham, 30 km north of Tehran, were α-pinene, α-fenchyl acetate, β-pinene and bornyl acetate (7). The different results of essential oils analysis demonstrated the effect of geographic origin on chemical composition of volatile oils.

Table 1. Volatile composition of essential oils from different parts of *Astrodaucus persicus*

No.	Compounds	KI	Flowers/fruits %	Stems/leaves %	Ripe fruits %	Root %	Methods of identification
1	α-Thujene	924	43.8	48.0	17.6	-	MS- KI
2	α-Pinene	932	20.9	27.7	14.3	0.6	MS- KI
3	α-Fenchene	945	-	9.2	-	-	MS- KI
4	Camphene	946	1.4	1.8	0.8	-	MS-KI
5	β- Fenchene	949	-	4.0	-	-	MS-KI
6	β-Pinene	974	21.3	-	56.9	0.3	MS- KI
7	β-Myrcene	988	2.0	-	2.4	0.4	MS-KI
8	α-Terpinene	1014	0.2	-	0.2	-	MS-KI
9	p-Cymene	1020	1.0	0.4	0.6	-	MS-KI
10	1,2,4-trimethylbenzene	1024	-	-	-	0.7	MS-KI
11	Limonene	1024	-	-	-	0.1	MS-KI
12	Eucalyptol	1026	-	-	-	0.2	MS-KI
13	cis-β-Ocimene	1032	0.1	-	0.1	-	MS- KI
14	Octatriene	1039	-	-	-	0.1	MS-KI
15	trans-β- Ocimene	1044	-	-	0.1	-	MS-KI
16	γ-Terpinene	1054	0.3	0.2	0.4	0.8	MS- KI
17	α-Terpinolene	1086	0.1	-	-	-	MS- KI
18	Linalool	1095	-	-	-	1.7	MS-KI
19	Perillene	1102	0.1	-	-	-	MS- KI
20	Alloocimene	1128	-	-	0.1	-	MS- KI
21	β-Citronellal	1148	-	-	0.4	-	MS- KI
22	α-Fenchyl acetate	1218	2.8	1.7	0.2	-	MS- KI
23	Bornyl acetate	1284	2.9	3.5	1.7	1.1	MS- KI
24	Sabinyl acetate	1289	0.1	-	-	-	MS- KI
25	Bicycloelemene	1330	-	-	-	0.5	MS-KI
26	α-Copaene	1374	-	-	0.1	-	MS- KI
27	β-Bourbonene	1387	-	-	0.1	1.0	MS- KI
28	Cedrene	1410	0.1	-	-	-	MS- KI
29	cis-α-Bergamotene	1411	-	0.1	-	-	MS- KI
30	trans-Caryophyllene	1417	0.1	-	-	33.5	MS-KI
31	β-Gurjunene	1431	-	0.1	-	-	MS- KI
32	Aromadendrene	1439	-	0.1	-	-	MS- KI
33	β-Farnesene	1440	-	-	-	7.2	MS-KI
34	Germacrene-D	1448	-	-	-	11.6	MS-KI
35	α-Humulene	1452	0.1	-	-	-	MS- KI
36	Bicyclogermacrene	1500	-	-	-	27.3	MS- KI
37	γ-Cadinene	1513	0.1	0.1	0.1	-	MS- KI
38	Myristicine	1517	-	-	1.2	-	MS- KI
39	δ-Cadinene	1522	-	-	0.1	-	MS- KI
40	Germacrene-B	1559	-	-	-	1.8	MS-KI
41	Spathulenol	1577	0.4	-	0.3	3.0	MS- KI
42	Caryophyllen oxide	1582	-	-	-	3.6	MS-KI
43	β-Eudesmol	1649	0.5	1.1	0.2	-	MS- KI
44	α-Eudesmol	1652	0.2	0.6	0.2	-	MS- KI
45	α-Cadinol	1652	-	-	-	1.2	MS-KI
46	Camazulene	1730	-	-	0.2	-	MS- KI
47	Hexadecanoic acid (Palmitic acid)	1959	-	-	0.6	3.3	MS- KI

Note: KI means Kovats Index

Table 2. Antioxidant activities and total phenols of *Astrodaucus persicus* (Boiss.) Drude

Samples	DPPH IC50 (µl/ml[a] or µg/ml[b])	FRAP (mmole Fe^{2+}/100 g)	Total phenol (mg GAE/g of sample)
Ripe fruits essential oil	>500	152.7±1.8	-
Flowers/fruits essential oil	>500	686.6±2.6	-
Stems /leaves essential oil	>500	152.0±4.8	-
Hexan fraction*	>1000	96.0±1.5	240.7±5.1
Chloroform fraction*	144.2	315.0±2.8	292.1±4.7
Ethyl acetate fraction*	99.2	677.0±5.7	872.8±3.4
Methanol fraction*	218	130.5±1.3	471.4±4.4
Total root extract	52.3	881.5±12.0	728.5±4.4
Vitamin E	14.1	313.7±2.2	-
BHA	7.8	880.3±6.4	-

Notes: * means different fractions of root extract; - means not examined; a for essential oils; b for fractions

Aerial parts essential oils of *A. persicus* demonstrated blue color while roots essential oil showed yellow color. Previous investigations recognized that the blue color of volatiles could be related to the structure of hydrocarbons and related sesqiuterpenes. Dehydrogenation of pure crystalline sesquiterpene alcohol, guaiol, to a blue hydrocarbon proved this hypothesis. Azulenes, the well-known blue structures formed a welcome addition to two dehydrogenation products, eudalene and cadalene. The formation of these naphthalene hydrocarbons has confirmed the mentioned hypothesis (19, 20). α-Eudesmol and β-eudesmol are sesquiterpene alcohols in blue aerial parts essential oils and did not exist in roots essential oil. The similar parts of eudesmol and guiaol are two cyclic rings and the branch of propan-2-ol which was not observed in other sesquiterpene alcohols in *A. persicus* roots essential oil including spathulenol, caryophyllene oxide and α-cadinol. Therefore dehydrogenation of α-eudesmol and β-eudesmol could be responsible for creation of blue color in aerial parts essential oils. The existence of camazulene (0.2%) could be a reason for intensification of blue color in ripe fruits essential oil.

Antioxidant and total phenols determination

Total root extract and EA fraction showed moderate activity of free-radical scavenging with IC_{50} of 52.3 and 99.2 µg/ml, respectively. The antioxidant activity of HE faction and all of aerial parts essential oil samples with DPPH method were negligible (IC_{50}>500 µg/ml). IC_{50} values of antioxidant capacities of three genera of Apiaceae family for example *Heracleum persicum* Desf., *Prangos ferulacea* (L.) Lindl. and *Chaerophyllum macropodum* Boiss. evaluated as 438, 242 and 623 µg/ml by DPPH method, respectively (21). *Caucalis platycarpos* L. and *Torilis leptophylla*, two near genera to Astrodaucus, demonstrated IC_{50} equal with 42.6 and 41.0 µg/ml based on DPPH radical scavenging method (22, 23). In comparison to other genera of Apiaceae, *A. persicus* root extract showed potent radical scavenging antioxidant activity.

Total antioxidant activity of aerial parts essential oils and root fractions were measured according to standard curve of $FeSO_4$ ($y = 0.001x + 0.049$, $r^2 = 0.925$). The greatest reducing capacity was belong to total root extract (881.5 mmol Fe^{2+}/ 100 g), which was comparable with BHA (880.3 mol Fe^{2+}/100 g) and more than vitamin E (313.7 mmol Fe^{2+}/100 g). Among volatile oils, the flowers/fruits essential oil showed potent reducing capacity (686.6 mmol Fe^{2+}/ 100 g) higher than vitamin E. In contrast, HE and ME fractions demonstrated the lowest antioxidant activity (96.0 and 130.5 mmol Fe^{2+}/100 g, respectively). The previous study on *Eryngium bourgatii* extract from Apiaceae family demonstrated the antioxidant effects equal to 59.8 mmol Fe^{2+} equivalents/g extract (24). Another investigation showed the reducing antioxidant effects of seeds of some Indian medicinal plants from Apiaceae including *Anethum sowa* Roxb., *Carum copticum* (L.) Benth. & Hook., *Coriandrum sativum* L., *Cuminum cyminum* L. and *Foeniculum vulgare* Mill. were equal to 175, 886, 33, 182 and 179 mmol Fe^{2+} /g dry weight, respectively (25). Our findings on *A. persicus* in comparison to previous works confirmed its high antioxidant activity.

The potent antioxidant activities of total root extract in comparison to its fractions demonstrated the synergist effect of compounds in various fractions for radical scavenging and reducing activities.

Total phenol content of samples were calculated based on gallic acid standard curve ($y = 0.007x$, $r^2 = 0.999$). EA fraction and total root extract showed the highest content of phenolic compounds among other samples (872.8 and 728.5 mg GAE/100 g sample). In previous study, total phenol content of *Cuminum cyminum* L. (Apiaceae) from Tunisia and India were measured as 1860 and 1450 mg of GAE/ 100 g dry weight, respectively (26). Another study on three medicinal Apiaceae species revealed total phenol

content of *Centella asiatica*, *Hydrocotyle bonariensis* and *H. sibthorpioides* as 72.09, 28.55 and 56.23 mg/100 g dry weight, respectively (27). *A. persicus* demonstrated moderate content of total phenols in comparison with another species of Apiaceae family.

Different fractions of root extract except HE fraction demonstrated negative correlation between IC_{50} of DPPH and FRAP antioxidant activities (y= -4.393x + 1049, r^2= 0.898). It means by increasing radical scavenging activity of fractions, the reducing capacity was increased so in root extract the same compounds were responsible for radical scavenging and reducing activity. There were not significant correlation between the amount of total phenols and DPPH antioxidant activity but there were proximate positive correlation between the total phenols and FRAP antioxidant activity in root fractions (y= -0.392x+612.6, r^2= 0.340 and y= 0.920x+188.8, r^2= 0.731, respectively). These correlations means by increasing the amount of phenols in fractions, the reducing antioxidant activity was increased so these compounds may involve in antioxidant activity by FRAP method. The previous study on burr parsley (*Caucalis platycarpos* L.) showed a significant positive correlation between flavonoids and phenolic acids content and antioxidant activities including lipid peroxidation, superoxide dismutase activity, metal ion chelating and reducing power assay, indicating the responsibility of these compounds for the antioxidant effectiveness (22). In another investigation on *Torilis leptophylla*, a significant but marginal positive correlation was found between total phenol content and EC_{50} values for DPPH, hydroxyl, phosphomolybdate and ABTS, whereas another weak and positive correlation was determined between total phenol content and EC_{50} values for superoxide anion and hydroxyl radicals. (23).

Conclusion

Chemical compositions of different parts essential oils of *A. persicus* from Kordestan Province were identified. α- and β- Eudesmoles were recognized as principle for formation of blue color in aerial parts essential oils. Root extract and flowers/fruits essential oil of *A. persicus* exhibited potent antioxidant activities and total phenols. These findings introduced *A. persicus* as a good potential source of natural antioxidants which may be related to its cytotoxic activity.

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