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Essential Oil of Arial Parts of *Adiantum capillus-veneris*: Chemical Composition and Antioxidant Activity

Laleh Khodaie,[1,2,*] Solmaz Esnaashari,[1] and Seddigheh Bamdad Moghaddam[1]

[1]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, IR Iran
[2]Department of Traditional Pharmacy, Faculty of Traditional Medicine, Tabriz University of Medical Sciences, Tabriz, IR Iran

*Corresponding author: Laleh Khodaie, Department of Traditional Pharmacy, Faculty of Traditional Medicine, Tabriz University of Medical Sciences, Tabriz, IR Iran. Tel: +98-4133337952, E-mail: khodaiel@gmail.com

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Abstract

Background: *Adiantum capillus-veneris* (Adiantaceae) is the only species of *Adiantum* genus growing in Iran. As a well-known plant in Iranian Traditional Medicine, to our knowledge, there has not been any report on essential oil constituents of this species as well as antioxidant activity evaluation of any plant belonging to *Adiantum* genus.

Objectives: This study aimed to determine phytoconstituents of *A. capillus-veneris* volatile oil and its antioxidant activity.

Materials and Methods: Essential oil obtained from arial parts of *Adiantum capillus-veneris* was analyzed by GC-Mass and its antioxidant activity was assessed by DPPH assay.

Results: Analyses of yellow colored essential oil yielded 88.22% of total oil with 67 components. Among identified phytochemicals, carvone was the main component (31.58%). Moreover, percentage of carvacrol (13.75%), Hexadecanoic acid (5.88%), Thymol (4.05%), Hexahydrofarnesyl acetone (3.16%) and n-nonanal (2.99%) were more than other identified constituents. RC50 of this volatile oil was 0.039 mg/mL.

Conclusions: Antioxidant activity of this essential oil could be somehow attributed to high contents of carvone, carvacrol and thymol. The volatile oil of this plant could have the potential of other biological activities, so further experimental investigations are recommended on this essential oil.

Keywords: DPPH Assay, Adiantaceae, Antioxidant Activity, *Adiantum capillus-veneris*

1. Background

Adiantum capillus-veneris belonging to Adiantaceae family is habitant in mild tropical and subtropical regions (1) consisting of 200 species widespread worldwide. *A. capillus- veneris* with Persian name of "parsiavashan" is the only species of *Adiantum* genus growing in Iran. As a well-known plant in Iranian Traditional Medicine, it is used as an expectorant, diuretic and lenient (2). Different phytochemicals such as terpenoids, flavonoids, phenyl ethanoids, steroids, alicyclic acids, lipids and long chain compounds have been isolated from different plants of *Adiantum* genus (3). In the recent years, there has been an increasing interest in medicinal plants essential oils demonstrated as natural antioxidants (4-7).

In previous studies, volatile oil composition of other species of *Adiantum* genus has been studied (1). Despite the fact, to our knowledge there has been no report on essential oil constituents of this species as well as antioxidant activity evaluation of any plant belonging to *Adiantum* genus.

2. Objectives

This study aimed to determine phytoconstituents of *A. capillus-veneris* volatile oil and its antioxidant activity.

3. Materials and Methods

3.1. Plant Material

Arial parts of *Adiantum capillus-veneris* were collected from Kordestan province of Iran, Hilu village in March 2012. For this collection, voucher specimens were deposited in the Herbarium of pharmacy faculty, Tabriz, Iran. Forty-five grams of ground and dried arial parts of *A. capillus- veneris* were subjected to hydrodistillation with distilled water (500 mL), using a Clevenger type apparatus for five hours. The oil was dried by anhydrous Na_2SO_4, measured and stored in dark glass bottles at 4°C for further analysis.

3.2. GC-MS Analysis

The volatile oil was analyzed using a Shimadzu GCMS-QP5050A gas chromatography mass spectrometer (GC-MS) fitted with a fused silica DB-1 capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness). Nitrogen was used as carrier gas at a flow rate of 1.3 mL/min. The oven temperature was kept at 50°C for 3 min, and programmed to rise to 270°C at a rate of 5°C/min and then kept constant for 9 minutes. The injector temperature was 250°C and

split ratio was adjusted at 1:15. The mass spectral (MS) data obtained at the following conditions; ionization potential 70 eV; ion source temperature 200°C; quadrupole temperature 100°C; solvent delay 3 minutes and EM voltage 3000 volts.

Identification of essential oil components was performed by comparison of their mass spectra with the NIST 21, NIST 107 and WILEY229 library, spectral data banks, which make available computer matching, direct comparison of retention times and mass spectral data with those of standard compounds as well as comparison of fragmentation patterns of mass spectra of unknown compounds with reported components. Kovats indices (KI) of phytoconstituents were obtained using standard n-alkanes (C8-C20) injection, under the same chromatographic conditions.

For quantitation (area %), the GC analyses were performed on a Shimadzu QP-5050A GC series apparatus fitted with a FID detector. The FID detector temperature was 300 °C. To obtain the same elution order as GC-MS, simultaneous auto-injection was performed on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of separated compounds were calculated from FID chromatograms.

3.3. DPPH Assay

Antioxidant activity of the essential oil of A. capillus-veneris was assessed using DPPH assay. Bleaching of purple colored chloroform, solution of 2, 2-diphenyl -1- pycryl hydrazyl (DPPH) (sigma) is the basis of this assay, so different sample solution series were prepared. Five milliliters of each concentration of essential oil was added to 5 mL of 0.004% chloroform solution of DPPH. After 30 minutes incubation of solutions at room temperature and bleaching of DPPH, absorption of samples was monitored at 517 nm against a blank. Inhibition of DPPH was calculated as RC50 that was extrapolated from dose-response curve. Tests were performed in duplicate (8-10). Trolax was used as a standard drug for assay.

4. Results

Volatile oil obtained by hydrodistillation of arial parts of *A. capillus-veneris* yielded a lemon yellow colored volatile oil of 0.15% (v/w). Table 1 demonstrates components of this volatile oil listed in order of their elution from DB1 column. Listed constituents were analyzed by GC-Mass both quantitatively and qualitatively. Sixty-seven components consisting 88.22% of total oil of *A. capillus- veneris* were identified. Among identified phytochemicals, carvone was the main compound (31.58%). Furthermore, percentage of carvacrol (13.75%), Hexadecanoic acid (5.88%), Thymol (4.05%), Hexahydrofarnesyl acetone (3.16%) and n-nonanal (2.99%) were more than other identified constituents. As seen in Table 1, phytochemicals in this essential oil were predominantly made up of monoterpenes (58.27%), also the monoterpene fraction was mainly consisted of oxygenated monoterpenes (57.22%). Moreover, sesquiterpenes (6.62%) and non-isoprenoid (23.33%) constituents existed in much lower percentages.

Table 1. Components of the Volatile Oil of *A. capillus-veneris*[a]

NO	Compound	% Area	RI[b]	Molecular Formula	Identification Method[c]
1	benzaldehyde	0.25	940	C_7H_6O	RI + MS
2	4-octen-3one	0.11	956	$C_8H_{14}O$	RI + MS
3	1- octen- 3oL	0.6	961	$C_8H_{16}O$	RI + MS
4	2-n-pentylfuran	0.08	980	$C_9H_{14}O$	RI + MS
5	n-octanal	0.27	992	$C_8H_{16}O$	RI + MS
6	1,8-cineol	0.19	1028.3	$C_{10}H_{18}O$	RI + MS
7	alpha-limonene	0.05	1032	$C_{10}H_{16}$	RI + MS
8	2-octenal (E)	0.17	1034	$C_8H_{14}O$	RI + MS
9	2-octen-1-ol	0.13	1048	$C_8H_{16}O$	RI + MS
10	1-octanol	0.32	1060	$C_8H_{18}O$	RI + MS
11	n-nonanal	2.99	1082	C_9H1_8O	RI + MS
12	L- linalool	0.83	1102	$C_{10}H_{18}O$	RI + MS
13	camphor	0.24	1126	$C_{10}H_{16}O$	RI + MS
14	trans-pinocarveol	0.07	1117	$C_{10}H_{16}O$	RI + MS
15	menthone	0.39	1152	$C_{10}H_{18}O$	RI + MS
16	borneol	0.15	1155	$C_{10}H_{18}O$	RI + MS
17	nonanol	0.21	1155	$C_9H_{20}O$	RI + MS
18	(-)-menthol	0.38	1166	$C_{10}H_{20}O$	RI + MS
19	terpineol-4	1.01	1168	$C_{10}H_{18}O$	RI + MS
20	myrtenal	0.18	1168	$C_{10}H_{14}O$	RI + MS

21	cis-dihydrocarvone	0.86	1206	$C_{10}H_{16}O$	RI + MS
22	cuminic aldehyde	0.82	1221	$C_{10}H_{12}O$	RI + MS
23	carvone	31.58	1223	$C_{10}H_{14}O$	RI + MS
24	nerol	0.18	1227	$C_{10}H_{18}O$	RI + MS
25	trans-anethol	1.95	1260	$C_{10}H_{12}O$	RI + MS
26	thymol	4.05	1268	$C_{10}H_{14}O$	RI + MS
27	carvacrol	13.72	1277	$C_{10}H_{14}O$	RI + MS
28	n-undecanal	0.14	1282	$C_{11}H_{22}O$	RI + MS
29	2,4-decadienal	0.45	1286	$C_{10}H_{16}O$	RI + MS
30	eugenol	0.7	1326	$C_{10}H_{12}O_2$	RI + MS
31	alpha-terpinyl acetate	0.44	1332	$C_{12}H_{20}O_2$	RI + MS
32	beta- bourbonene	0.1	1380	$C_{15}H_{24}$	RI + MS
33	delta-selinene	0.3	1383	$C_{15}H_{24}$	RI + MS
34	dodecanal	0.22	1383	$C_{12}H_{24}O$	RI + MS
35	n-tetradecane	0.9	1403	$C_{14}H_{30}$	RI + MS
36	alpha- Ionone	0.27	1408	$C_{13}H_{20}O$	RI + MS
37	geranyl acetate	0.56	1428	$C_{13}H_{22}O$	RI + MS
38	alpha-bergamotene	0.99	1436	$C_{15}H_{24}$	RI + MS
39	(Z)- beta-Farnesene	0.26	1450	$C_{15}H_{24}$	RI + MS
40	alpha.-Santalene	0.13	1460	$C_{15}H_{24}$	RI + MS
41	beta-ionone	0.97	1461	$C_{13}H_{20}O$	RI + MS
42	alpha-curcumene	0.36	1474	$C_{15}H_{22}$	RI + MS
43	E-2-tridecenal	0.13	1478	$C_{13}H_{24}O$	RI + MS
44	alpha-murolene	0.28	1499	$C_{15}H_{24}$	RI + MS
45	n-pentadecane	1.7	1503	$C_{15}H_{32}$	RI + MS
46	calamenene	0.26	1516	$C_{15}H_{22}$	RI + MS
47	delta-cadinene	0.22	1520	$C_{15}H_{24}$	RI + MS
48	endo-1-bourbonanol	0.5	1537	$C_{15}H_{26}O$	RI + MS
49	caryophyllenyl alcohol	0.62	1566	$C_{15}H_{26}O$	RI + MS
50	(+)- spathulenol	0.39	1570	$C_{15}H_{24}O$	RI + MS
51	cubenol	0.15	1624	$C_{15}H_{26}O$	RI + MS
52	beta- tumerone	0.12	1634	$C_{15}H_{22}O$	RI + MS
53	cadalin	0.28	1655	$C_{15}H_{18}$	RI + MS
54	jatamansone	0.22	1658	$C_{15}H_{26}O$	RI + MS
55	2-pentadecanone	0.1	1674	$C_{15}H_{30}O$	RI + MS
56	hexadecanal	0.24	1696	$C_{16}H_{32}O$	RI + MS
57	heptadecane	0.5	1703	$C_{17}H_{36}$	RI + MS
58	tetradecanoic acid	0.16	1713	$C_{14}H_{28}O_2$	RI + MS
59	octadecane	0.08	1803	$C_{18}H_{38}$	RI + MS
60	hexahydrofarnesyl acetone	3.16	1834	$C_{18}H_{36}O$	RI + MS
61	Z-9-octadecenal	0.32	1876	$C_{18}H_{34}O$	RI + MS
62	octadecanoic acid, methyl ester	0.2	1911	$C_{19}H_{38}O_2$	RI + MS
63	caprylic ether	0.56	1914	$C_{16}H_{34}O$	RI + MS
64	hexadecanoic acid	5.88	1920	$C_{16}H_{32}O_2$	RI + MS
65	phytol	2.1	2106	$C_{20}H_{40}O$	RI + MS
66	linoleic acid	0.72	2088	$C_{18}H_{32}O_2$	RI + MS
67	oleic Acid	0.76	2097	$C_{18}H_{34}O_2$	RI + MS

[a] Total compounds = 76; Oxygenated monoterpenes = 57.22%; Monoterpene hydrocarbons =0.05%; Oxygenated sesquiterpenes = 2.1%; Sesquiterpene hydrocarbons = 4.52%; Non-terpene hydrocarbons = 23.33%; Total identified = 88.22%.
[b] Compounds listed in order of elution from a DB-1 column.
[c] Identification method (RI, Retention indices; MS, Mass spectroscopy).

Antioxidant activity of *A. capillus-veneris* volatile oil was assessed by DPPH assay, in which the basis is decolorization of DPPH and measurement of the absorbance of test tubes containing different concentrations of essential oil. RC50 of this volatile oil was 0.039 mg/mL. RC50 of Trolax as positive control was 3.07×10^{-3} mg/mL.

5. Discussion

Free radicals, defined as atoms or molecules with odd number of electrons, exist in many chemicals and have been suggested to contribute in cell structure and tissue damage, which eventually cause human disease and aging mechanism (11-13). Antioxidants protect body against free radical-mediated and oxidative damage by scavenging free radicals (14), so increasing antioxidant intake can be a preventive factor for lowering oxidation (15, 16). Nowadays, there is a growing interest for identifying antioxidants from natural sources due to a new trend for substitution of synthetic antioxidants with natural ones, which exist in medicinal plants used in traditional medicine (17,18). Essential oils obtained from medicinal plants can be used as natural antioxidants (5). *A. capillus- veneris* is a fern widely used for various diseases in Iranian traditional medicine (19). According to the literature, isolated phytochemicals of A. capillus-veneris were triterpenoidal compounds belonging to adiantane and filicane groups and identified as isoadiantone, isoadiantol-B, 3-methoxy-4-hydroxyfilicane and 3, 4-dihydroxyfilicane as well as three flavonoids of quercetin, quercetin-3-O-glucoside and quercetin-3-O-rutinoside (rutin). Modern pharmacological studies revealed antimicrobial, antifungal, anti-implantation, antioxidant, anti- inflammatory, anti-diabetic and anti-nociceptive effects of this plant (20-24). This paper discussed the above purposes since no research has been performed concerning chemical constituents and antioxidant activity of this species essential oil.

Table 1 demonstrates major constituents of the essential oil of *A. capillus-veneris*. As shown in Table 1, the main constituent of this volatile oil was carvone (31.58%). Other major constituents were carvacrol (13.72%), hexadecanoic acid (5.88%), thymol (4.05%), Hexahydrofarnesyl acetone (3.16) and n-nonanal (2.99%). In another study conducted to determine constituents of the essential oil of *Adiantum flabellulatum*, the major constituents identified from roots of this plant were n-decanoic acid (11.44%), 6,10,14-trimethyl-2-pentadecanone (11.23%), diethyl phthalate (8.63%), and nonanoic acid (6.15%). The major constituents identified from leaves of *A. flabellulatum* were n-decanoic acid (11.77%), 2-isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7- octahydronaphthalene (10.63%), [1R-(1α,7β,8α]-1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-naphthalene (9.88%), α -panasinsen (8.11%), 4-tetradecyne (6.63%), β -pinene (5.16%) and nonanoic acid (4.01%) (1). Moreover, in another article about phytoconstituents of essential oil of *Adiantum edgeworthii*, n-nonanal was the main constituent in leaves, while 2,6-di-tert-butyl p-cresol was the main active constituent in the roots of this plant (25). These results indicate fundamental differences among essential oils of different species of *Adiantum* genus.

Antioxidant activity of volatile oil of *A. capillus-veneris* was assessed by DPPH assay, a simple and reliable experiment to screen antioxidant agents. Findings of this assay showed that the volatile oil of this plant has the potential of scavenging free radicals. Phytochemicals of this essential oil indicated that this effect could be somehow attributed to high contents of carvone (26), carvacrol and thymol. Thymol and carvacrol as phenolic compounds are responsible for radical scavenging activity of many essential oils, which contain these two compounds (27).

This study determined chemical composition and antioxidant activity of the essential oil of *A. capillus- veneris*. Phytochemicals responsible for its radical scavenging activity were discussed. In conclusion, the volatile oil of this plant could have the potential of other biological activities, so it is recommended to perform further experiments on this essential oil.

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Footnote

Authors' Contribution: Laleh Khodaie abstracted and analyzed data, wrote and prepared the manuscript and is guarantor. Solmaz Esnaashari developed the original idea and Seddigheh Bamdad Moghaddam obtained essential oil.

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