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Chemical composition of the essential oil of *Artemisia austriaca* JACQ. growing wild in Iran

[İran'da doğal ortamda büyüyen *Artemisia austriaca* JACQ bitkisinin esansiyel yağına ait kimyasal yapı]

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ABSTRACT

Objective: The genus *Artemisia* (Asteraceae) comprises approximately 400 species of commonly perennial and fragrant herbs distributed in the northern temperate region of the world. *Artemisia austriaca* JACQ. is one of the most common species of the genus distributed from central Europe to Siberia, Turkey, and Iran. In Iran, the plant is indigenous to East Azerbaijan and Ardabil province. In the present study, the essential oil composition of the aerial part of *Artemisia austriaca* and some allelopathic potential of the oil were studied.

Methods: The essential oil of the plant was obtained by hydrodistillation using a clevenger type apparatus. After dehydratation, the essential oil was analyzed by GC and GC-MS.

Results: The results showed that the camphor (15.88%), 1,8-cineole (10.75%), camphene (3.53%) and beta-fenchyl alcohol (3.03%) were the main components among 51 constituents characterized in the oil. The plant essential oil showed significant anti-sclerotinia and phytotoxic activity.

Conclusion: Our findings indicated that there is a high level similarity between the major compound of essential oil of *A. austriaca* samples collected from northwest of Iran, Ardabil, and Turkey. Accordingly, the two samples may represent the same chemotype. On the other hand, it could be concluded that plant essential oil could play an allelopathic role for plant.

Key Words: *Artemisia austriaca*, essential oil, allelopathy

Conflict of Interest: There is no conflict of interest among the authors who contributed to the present study.

ÖZET

Amaç: *Artemisia* (Asteraceae) sınıfı yaklaşık 400 türden oluşan, dünyada kuzey iklim bölgesine dağılmış çoğunlukla çok yıllık, sürekli ve güzel kokulu şifalı bitkidir. *Artemisia austriaca* JACQ., Orta Avrupa'dan Sibirya, Türkiye ve İran'a kadar dağılan sınıfın en bilinen türüdür. Bitki, İran'da Batı Azerbeycan ve Ardabil bölgesine özgüdür. Bu çalışmada, *Artemisia austriaca*'nın toprak üzerinde olan kısımlarının esansiyel yağ bileşeni ve yağın bazı allelofatik potansiyeli çalışıldı.

Metod: Bitkiye ait esansiyel yağ, clevenger tip aparat kullanılarak hidrodistilasyon ile elde edildi. Dehidratasyon sonrası esansiyel yağ GC ve GC-MS ile analiz edildi.

Bulgular: Sonuçlar, yağda karakterize edilen 51 bileşen içinden, kafur (camphor; %15.88), 1,8-sineyol (%10.75), halis neftyağı (camphene; %3.53) ve beta-fensil alkol (%3.03)'ün ana bileşen olduğunu gösterdi. Bitkiye ait esansiyel yağ belirgin anti-sklerotinia ve fitotoksik aktivite gösterdi.

Sonuç: Bulgularımız İran Ardabil kuzey bölgelerinden toplanan *A. austriaca* örneklerindeki esansiyel yağ bileşeni ile Türkiye örnekleri arasında yüksek düzeyde benzerlik olduğunu işaret etti. Bu nedenle her iki örnek de aynı kemotipi temsil edebilir. Diğer taraftan sonuç olarak bitkiye ait esansiyel yağın, bitkiler için allelopatik rol oynayabileceği söylenebilir.

Anahtar Kelimeler: *Artemisia austriaca*, esansiyel yağ, allelopati

Çıkar Çatışması: Çalışmaya katılan yazarlar çıkar çatışması bildirmemiştir.

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Introduction

In the flora of Iran, the genus *Artemisia* has been introduced by 64 species. These plants are annual or perennial herbs with pleasure odor distributed in all over parts of Iran. Afsantin is a common name for the *Artemisia* species in Iran where there are used as medicinal plants [1]. Aerial parts of some *Artemisia* species have been used for their spasmolytic activity in Iranian folk medicine [2].

A literature review showed that some substances from the *Artemisia* genus indicated various biological activity. They may exhibit antifungal, antiviral, antitumor, antipyretic, antioxidant, anticoagulant, antihemorrhagic, and antihepatitic activity [3-6].

Artemisia austriaca is regarded as a perennial member of the genus distributed in northwest of Iran in Azerbaijan province. However, the plant is widespread from Eastern Europe to Anatolia and Caucasia steppes [7]. In the north west of Iran, the plant is used as a medicinal plant for treatment of gastric disorders. It is also used as an animal fodder in the plateau regions of northwest of Iran. This aromatic herbs has branched stems and pinnatisect leaves covered with whitish to silvery piloses. Its reddish to yellowish flowers appears in summer [1]. To our knowledge, no previous study on essential oil composition of A.austriaca from Iran has been reported.

Material and Methods

Plant material

The aerial parts of *A. austriaca* at flowering stage were collected in July 2011, from the Ardabil province, Iran. The voucher specimen (No.1391-0) was deposited in the herbarium of Faculty of Sciences, University of Mohaghegh Ardabili.

Essential oil isolation

Aerial parts of the plant (100 g) were crushed and subjected to water distillation for 3 h using a Clevenger apparatus. The essential oils was dried over anhydrous sodium sulfate and stored at 4°C in dark.

Essential oil analyses

The oils were diluted with n-hexane and then analyzed by a GC-MS apparatus. The analysis was carried out on a Thermoquest-Finnigan Trace GC/MS instrument equipped with a HP-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was programmed to increase from 50ºC to 320ºC at a rate of 4ºC/min and finally held for 7 min; transfer line temperature was 250ºC. Helium was used as the carrier gas at a flow rate of 0.8 mL/min with a split ratio equal to 1/60. The quadruple mass spectrometer was scanned over the 35-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 µA.

Identification of essential oil components

Identification of compounds was based on the comparison of their retention indices (RI) with those reported in the literatures [8] and their mass spectrum with the Wiley library (Wiley 7.0), as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [8].

Phytotoxic assay

Phytotoxic assay was carried out by Lettuce (*Lactuca sativa* L. cv Varamin) seeds with evaluating response of seed germination, shoot and root elongation of seedlings to different concentrations of the essential oil. The oil was dispersed as an emulsion in water using Tween 20. Seven concentrations of the oil (0.0001, 0.001, 0.01, 0.1, 1 and 10 mg mL-1) were obtained by dilution of the emulsions with deionized water. All seeds were surface sterilized with sodium hypo chloride (1%). Four replicates, each of 25 seed, were prepared for each treatment using sterile petri dishes (90 mm) lined with one sterile filter paper (Whatman, number 2). A volume of 5 ml of different concentrations of the oils was added to each petri dish. Prepared plates were then placed in a germination cabinet at 25°C in the dark. After 1 week, in the each treatment, germination percentage was determined and root and shoot length was measured [9].

Antifungal activity assay

The antifungal activity assay was performed on *Sclerotinia sclerotiorum* (Lib.) de Bary fungus that causes stem rot in many plants such as Rapeseed, Sunflower and Lettuce and it is one of the most prevalent plant pathogens. In this study, an isolate of *Sclerotinia sclerotiorum* from rapeseed was used. The assay was assessed by means of combination with the medium (PDA) at 4 concentrations of the essential oil (0.01, 0.1, 1 and 10 mg/mL). The PDA was poured into Petri plates that were then inoculated with 4 mm plugs from 7 days old cultures. The control experiments had distilled water in place of essential oil. The cultures were incubated at 25ºC for 7 days. The diameter of the radial growth of the fungi was measured at the end of incubation period and then used to determine the percentage inhibition of each concentration using the formula:

Mycelia growth inhibition (%) = [(dc-dt)/dc]×100(%)

Where Dc = average diameter of fungal colony in the control and

Dt = average diameter of fungal colony in the treatment group.

Amphotericin B was applied as positive control. The MIC values of the oil and Amphotericin B against the test microorganism were determined by the Agar dilution method [10].

Statistical analysis

In all assays, SPSS 14 software was used for statistical analysis. The normality assumption of data was evaluated by Kolmogorov and Smirnov test. Analysis of variance (ANOVA) followed by Duncan test was used to see the difference amongst various groups. The significance level was set at $p \leq 0.05$.

Table 1. Chemical composition of the essential oil of *Artemisia austriaca* aerial parts

No	Compound	Percentage	R_t	R_{ts}	RI
1	2-methylbutan-1-ol	1.51	3.47	2.85	792
2	Hexanal	0.39	4.81	3.99	855
3	Delta-cadinene	0.21	4.82	29.72	1523
4	1,3-cyclopentadiene	0.50	5.26	19.62	1290
5	Nonene	0.28	7.10	13.94	1162
6	Heptanal	0.35	7.31	15.26	1190
7	Alpha-thujene	0.50	7.85	5.62	0930
8	Alpha-pinene	3.06	8.01	5.85	939
9	Camphene	3.56	8.30	6.26	954
10	Verbenene	0.26	8.36	6.67	968
11	Sabinene	1.48	8.70	6.91	975
12	Beta-pinene	3	8.76	7.04	979
13	1-octene-3-ol	0.56	8.90	7.04	979
14	Beta-myrcene	0.74	8.97	7.43	991
15	Alpha-phellandrene	0.53	9.19	8.96	1030
16	Alpha-terpinene	3.11	9.39	8.30	1017
17	1,8-cineole	10.75	9.74	8.76	1031
18	Gamma-terpinene	2.25	10.02	9.77	1060
19	Trans-sabinene hydrate	1.22	10.15	10.20	1070
20	Alpha-terpinolene	1.09	10.40	10.92	1089
21	Cis-sabinene hydrate	0.50	10.57	10.20	1070
22	Amyl-isovalerate	1.36	10.61	–	–
23	Camphor	15.88	11.37	13.28	1146
24	Borneol	9.18	11.59	14.29	1169
25	Beta-fenchyl alcohol	6.14	11.75	16.57	1220
26	Terpinene-3-ol	0.41	11.88	14.66	1217
27	Carveol	0.52	11.99	16.44	1217
28	Cis-carveol	0.58	12.10	16.94	1229
29	Geraniol	0.35	12.29	17.95	1253
30	Chrysanthenyl acetate	1.70	12.40	18.51	1265
31	Alpha-terpinyl acetate	1.34	12.77	20.96	1318
32	2,4-decadienal	0.22	12.89	20.87	1377
33	Eugenole	0.88	13.32	22.70	1359
34	Geranyl acetate	0.24	13.46	23.70	1321
35	Bornyl acetate	0.36	13.58	19.62	1289
36	Trans- caryophellene	0.48	13.99	25.36	1419
37	Alpha-gurjunene	0.47	14.44	25.00	1410
38	Germacrene D	0.35	14.51	28.15	1485
39	Beta-selinene	0.90	14.58	28.37	1490
40	Beta-humulene	0.24	14.64	26.16	1439
41	Delta-cadinene	0.21	14.82	29.72	1513
42	Caryophelene oxide	0.67	15.12	32.16	1583
43	Trans-davanone	0.24	15.29	31.46	1566
44	Spathulenol	0.77	15.33	31.96	1578
45	Azulene	0.43	15.68	20.11	1298
46	Copaene	1.58	15.78	23.49	1377
47	Caryophylla-dien-5-beta-ol	0.34	15.82	34.40	1641
48	Beta-eudesmol	1.18	15.94	34.79	1651
49	Cis-davanone	0.24	15.99	32.37	1588
50	Vulgarol B	0.48	16.19	34.21	1651
51	1,6-octadecadiene oxide	1.61	19.53	–	–

Rt = observed retention time (min); Rts = standard retention time; RI = Adams standard retention indices.

Table 2. Chemical class distribution of the essential oil components of *Artemisia austriaca* aerial parts

Compound class	Percentage
Sesquiterpens	4.87
Oxygnatedsesquiterpens	3.71
Monoterpenes	19.55
Oxygnatedmonoterpenes	46.33
Hydrocarbonos	4.71
Esters	4.71

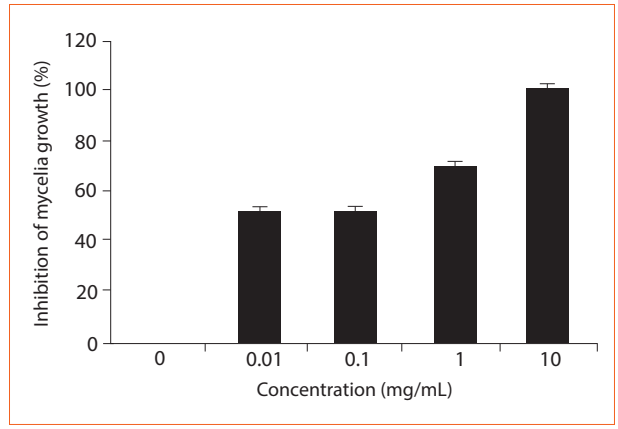

Figure 1. Antifungal activity of the essential oil of *Artemisia austriaca* on Sclerotinia sclerotiorum.

Results

The aerial parts of *A. austriaca* yield 0.7 % (v/w) of a light blue color oil. The GC-MS analysis of the oil revealed 51 compounds, totally. The main components of the oil were characterized as camphor (15.8%), 1,8 cineole (10.7%), borneol (9.1%), camphene (3.5%) and beta-fenchyl alcohol (3%) (Table 1). The major compound class of the essential oil was: oxygenated monoterpenes (46.3%) and monoterpenes (19.5%) (Table 2).

The results of antifungal assay showed that the essential oil of *A. austriaca* exhibited considerable inhibitory effects against *Sclerotinia sclerotiorum*, a common plant pathogen fungus. At the concentration of 0.01 mg/mL, the oil stunts the fungus mycelia growth to 50% than control (Fig. 1). The MIC value of amphotericin B, a positive control, for tested microorganism was calculated as 0.025 mg/mL, as well as. Thus, it is concluded that the A. austriaca oil effect against *Sclerotinia sclerotiorum* could be regarded in a strong range.

Our results also indicated that the *A. austriaca* oil displayed modest phytotoxic activity. The oil at the concentrations higher than 1 mg/mL significantly reduced seed germination, shoot and root length of lettuce (Table 3).

Discussion

A previous report has been presented chemical composition of *A. austriaca* oil collected from central parts of Iran where the main constituents of the oil were as camphene (35.7%) [11]. There are also reports on essential oil composition of the plant collected from Turkey introduced camphor (45.5%) as major compound of the oil [12]. These results along with those from the present study revealed that there is a considerable similarity between the profile and major compounds of essential oil of *A. austriaca* samples collected from Turkey and Iran. Therefore, it is concluded that the same chemotype of *A.austriaca* might be distributed from central Iran to Turkey.

A literature review showed that the essential oil compositions of many *Artemisia* species have been previously investigated. According to major compounds of essential oils, it is possible to divide *Artemisia* species into two groups and a few subgroups:

1) Species producing oxygenated monoterpenes as major constituents of the oil

i) Species with camphor and 1,8-cineole as characteristic compounds, such as *A. oilveriana* [13], *A. spicigera* [12], *A. deserti* [13], *A. annua* [14], *A. rigid* [15], *A. radicus* [15], *A. incana* [16], and *A. austriaca* (present work).

ii) Species with thujone as major component, such as *A. scoparia* [17] and *A. arborescens* [18].

2) Species containing mainly sesquiterpene hydrocarbon, germacrene D, such as *A. vulgaris* [19] and *A. parviflora* [20].

Table 3. Phytotoxic activity of essential oils of *Artemisia austriaca* aerial parts

Concentration (mg mL-1)	Germination (%)	Shoot Length (cm)	Root Length (cm)
0	84±0.28^{a}	2.62±0.15^{a}	4.78±0.36^{a}
0.0001	78±0.64^{ab}	2.68±0.03^{ab}	5.43±0.39^{ab}
0.001	72±1.22 ^{ab}	2±0.07^{ab}	4.5±0.17^{ab}
0.01	78±1.04^{ab}	2.84±0.03^{ab}	4.98±0.20^{ab}
0. 1	64.0±0.62^{ab}	2.45±0.21^{ab}	3.39± 0.41^{ab}
1	64±0.82^{b}	2.32±0. 3^{b}	3.90±0.42^{ab}
10	67±2.09^{b}	1.26±0.17^{c}	1.98±0.20^{c}

* Mean values in the same column followed by the same letter (a, b, c) are not significantly different at p ≤0.05 according to the Duncan test. Totally sample size for each concentration was 100.

Due to significant antifungal and phytotoxic properties of *A. austriaca* oil, it can be assumed that the oil might play an ecological role for plant and can be considered as allelopathic agent. In the recent years, allelopathy is commonly defined as the ability of a plant to suppress the growth of neighboring plants or reduce activity of a pathogen microorganism or herbivorous insect by producing allelochemicals. In fact, allelopathy in plants is regarded as a natural strategy protecting plants against environmental enemies and competing plants. In the last decades, the application of synthetic toxins for control of weeds, pests, and plant disease has caused serious environmental problems. Thus, allelopathy interactions between plants and other organisms may become an alternative to synthetic herbicides and other pesticides [21].

References

[1] Rechinger KH, Hedge IC. Flora Iranica, Vol:158. 1986; Akademische Druke Verlgsanstalt, Graz.

[2] Zargari A. Medicinal Plants, Vol3. 6th Ed. 1996; pp. 97-98, Tehran University Press, Tehran.

[3 Shafi PM, Narnbiar MKG, Clery RA, Sarma YR, Veena SS. Composition and antifungal activity if oil of *Artemisia nilagrica* (Clarke). Parnp 2004; 16: 377-379.

[4] Mirjalali MH, Tabatabaei SM, Hadian J, Nejad-Ebrahimi S, Sonboli A. Phenological variation of the essential oil *Artemisia Scoparia Waldst*. Et kit from Iran. J Essent Oil Res. 2007; 19: 326-329.

[5] Buritis M, Asrers K, Bucar F. The antioxidant essential oil of *Artemisia abssinica* and *Juniperus procera.* Phytother Res. 2001; 15: 103-108.

[6] Iuteau F, Bessiere IM, Masotti V, Dherbomez M, Viano I. Antibacterial and antioxidant activities of *Artemisia* arma essential oil. Fitoterapia 2002; 73: 532-535.

[7] Davis PH. Flora of Turkey and the East Aegean Islands. Vol 5. 1975; pp. 311-313, Edinburgh University Press, Edinburgh.

[8] Adams RP. Identification of essential oil component by gas chromatography/quadrupole mass spectroscopy 2004; pp.10-360. Allured Publishing Corporation, Illinois.

[9] Razavi SM, Zarrini G, Molavi G, Ghasemi G. Bioactivity of Malva Sylvestris L., a Medicinal Plant from Iran. Iran J Basic Med Sci. 2011; 14: 574-579.

[10] Razavi SM, Zarrini G. Bioactivity of aviprin and aviprin-3''-O-glucoside, two linear furanocoumarins from Apiaceae. Russ J Bioorg Chem. 2010; 36: 359-362.

[11] Costa R, Defina MR, Valentino MR, Rustaiyan A, Dugo P, Dugo G, Mondello L. An investigation on the volatile composition of some *Artemisia* species from Iran. Flav Frag J. 2009; 24: 75-82.

[12] Guvenalp Z, Cakir A, Hamandar M, Gleispach H. The essential oil of *Artemisia austriaca* Jacq and *Artemisia spicigera* C. Koch. From Turkey. Flav Frag J. 1998; 13: 26-28.

[13] Rustaiyan A, Komeilzadeh H, Masoudi S, Monfared A, Kardar M, Shahgholi A. Composition of rigid volatile oil of *Artemisia deserti* and *Artemisia oliveriana* J. Gayex. J Sci. 2000; 11: 213-215.

[14] Holm Y, Laakso I, Hiltunen R, Galambosi B. Variation in the essential oil composition of *Artemisia annua* L. of different origin cultivated in Finland. Flav Frag J. 1997; 12: 241-246.

[15] Atuzhanova GA, Dembitskii AD, Yakovleva TD, Ishmuvatova M, Mikhailova VG, Adekenov SM. Composition of the essential oils of *Artemisia radicans* and *A. rigid.* Chem Nat Comp. 1999; 35: 427-429.

[16] Cetin B, Ozek H, Cakir A, Mete E, Tosun M, Ozturk E, Polat T, Kandemir A. Chemical composition of hydrodistillated essential oil of *Artemisia incana* (L.) Drude and antimicrobial activity against foodborne microorganisms. Chem Biodivers. 2009; 6: 2302-2308.

[17] Mirjalali MH, Tabatabaei SM, Hadian J, Nejad-Ebrahimi S, Sonboli A. Phenological variation of the essential oil *Artemisia Scoparia Waldst*. Et kit from Iran. J Essent Oil Res. 2007; 19: 326-329.

[18] Beyrouthy MEI, Apostolides NA, Labaki M, Cazier F, Najma S, Aboukais A. Chemical composition of the essential oil of the *Artemisia arborescens*, growing wild in Lebanon Leb Sci J. 2011; 12: 71-78.

[19] Judzentiene A, Buzeytle J. 2006. Chemical composition of essential oils of *Artemisia vulgaris* L. (mugwort) from north Lithuania. Chemija 17: 12-15.

[20] Rana VS, Juyal JP, Blazquez MA, Bodakhe SH. Essential oil composition of *Artemisia parviflora* aerial parts. Flav Frag J. 2003; 18:342-344.

[21] Razavi SM. Plant coumarins as allelopathic agents. Int J Biol Chem. 2010; 51: 86-90.