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"Research Note"

THE CONSTITUENTS OF ESSENTIAL OILS OF *FERULAGO ANGULATA* (SCHLECHT.) BOISS AT TWO DIFFERENT HABITATS, NEVAKOH AND SHAHOO, ZAGROSS MOUNTAIN, WESTERN IRAN*

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Abstract – *Ferulago angulata* (Apiaceae), a medicinal plant of western Asia, contains essential oils that are used as a food preservative. This study examined and compared the composition of leaf oil with seed oil and of the oils from two different habitats (one from Nevakov and the other from Shahoo). The oil yield from seed was 5-fold that from leaves (3.2%/100g compared to 0.63%/100g). Cis-ocimene was the major constituent of the seed oil from both regions (64.8% and 76.11%) and a prominent constituent (>20% of the total oil) of the leaf oils of both habitats. α-Pinene was the next main component (7-27%) of all 4 oils. Seed oils, with one major component (cis-ocimene), differed from the leaf oils, which were composed mostly of 3 components (α-pinene, cis-ocimene, & germacrene D). Distinctions between the oils of the two habitats were less marked than the leaf-oil/seed-oil differences; the cis-ocimene content was higher and α-pinene was less in both seed- and leaf-oils of the Shahoo habitats than the Nevakoh ecotype; trans-verbenol was absent from the Shahoo leaves, but reached a content of 5.8% in Nevahoh leaf-oil. Further distinctions were found in the content/presence/absence of 20-30 minor components of the oils.

Keywords – Ferulago angulata, medicinal plant, essential oil

1. INTRODUCTION

The *F. angulata*. plant (known in Iran as Chavir) is a perennial shrub 60-150 cm tall, grows at altitudes of 1900- 3200m (above sea level) [1, 2] and is distributed in the east of Turkey, North Iraq and Iran. *F.angulata* has two subspecies: subsp. *angulata* (Schlecht.) and subsp. *carduchorum* (Boiss & Hausskn.). The species *angulata* is widespread in all of the above countries. In other ecotypes of Iran the oil extract analysis of this subspecies has been performed by other researchers [3], but the subspecies *carduchorum* is relict endemic to Shahoo (Zagross Mountain, western Iran) [4, 5]. The oil has long been used as an additive to edible oil (eg. Rughan Kermanshahi) and as a food preservative [6].

The seed and leaves of *F.angulata* were sampled from two different ecotypes, one ecotype located on Shahoo Mountain (sub sp. *Carduchorum*) and the other on Nevakoh Mountain (subsp. *angulata*), both in Kermanshah state, Zagross Mountain, western Iran. The oils were hydro distilled and analyzed by GC and GC/Mass to determine their physical properties and the chemical constituents of the oils of *F.angulata* leaves with oils from the seeds in two regions *(two subspecies)*.

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2. EXPERIMENTAL

a) Plant material

Fresh *F.angulata* were leaves gathered and air dried in May, 2004 and the seeds collected in October, 2004 from both habitats (Shahoo and Nevakoh Mountains), Kermanshah Province western Iran.

b) Extraction procedure

100g of each, leaves and seeds of *F.angulata* were powdered, mixed with 1200 ml and 1000ml (respectively) of distilled water and the essential oils hydro distilled in a Clevenger-type (Clevenger 1928) apparatus according to the British method for 3 h. The essential oils were dried over anhydrous Na_2SO_4 and stored at 4˚C in the dark. Essential oil yields were 0.63 ٪ (w/v) for leaves and 3.2٪ (w/v) for seeds based on the dry weights of the samples.

c) Gas chromatography (GC)

A Thermoquest-Finnigan Trace GC equipped with a DB-1 fused silica column (60 m×0.25 mm i.d., film thickness 0.25 μm) was used for GC analysis. Nitrogen was the carrier gas, at a constant flow of 1.1 ml/min. The oven temperature was held at 60°C for 1 min, then programmed to 250°C at a rate of 4°C /min, and then held for 10 min. The injector and detector (FID) temperatures were kept at 250°C and 280°C, respectively.

d) Gas chromatography-mass spectrometry

GC-MS analysis was carried out on a Thermoquest-Finnigan Trace GC-MS instrument equipped with a DB-1 fused silica column (60 m×0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 60°C to 5°C/min and samples were injected at 250°C. Spectra were recorded in the scan mode at 70 eV.

e) Quantitative analysis

The constituents were identified from calculations of their retention indices under temperature-programmed conditions for *n-alkanes* (C_6–C_{24}) on a DB-1 and DB-Wax columns. Individual compounds were identified by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with authentic compounds. Identifications were confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature [7]. Quantitative data was obtained from FID area percentages (without the use of correction factors).

3. RESULTS AND DISCUSSION

The major constituents of the essential oil were found to be monoterpene hydrocarbons and sesquiterpene hydrocarbons (Table 1). Clear differences in the constituent compounds were observed in oils from the leaves and seeds collected from the two different habitats (Nevakoh and Shahoo Mountains). The major constituents of leaf oils (Table 1) were: α-pinene (27٪, 25٪), cis-ocimene (22٪, 27٪), and bornyl acetate (8.5٪, 3.9٪). The major constituents in the seed oils were cis-ocimene (64٪, 76٪) and α-pinene (15٪, 7.3٪). The percentage yield of oil was 5 times greater from seed than from leaves. Whereas a single component cis-ocimene constituted most of the oils from seeds (~65-74%), the leaf oils contained only three components, which exceeded 20% of the oil (α-pinene, cis-ocimene, & germacrene D). Apart from a much lower germacrene D content in Nevakoh leaf oil than in Shahoo leaf oil, differences between the oil from the two ecotypes involved only the minor oil components. The minor constituents α-thujene, 3-carene, α-

humulene, allo-aromadendrene, 1,3,8-p-menthatriene, elemene (γ and δ), and γ-muurolene were found only in Shahoo province extracts. Ortho-cymene, linalool, cis-verbenol, trans-verbenol, cadrol, methyl euginol, ipsdienol, p-mentha-1,5-dien-8-ol and bicyclogermacrene were detected only in Nevakoh province extracts.

In the case of the seed oils, the major component, cis-ocimene, had an 11% higher content in the Shakoo oil than in the Nevakoh oil, whereas the component with the next greatest content, α-pinene, had a content 8% higher in the Nevakoh oil. Cis-epoxy ocimene, cis-verbenol, p-mentha-1,5-dien-8-ol, thymol, myretenyl acetate, benzyl isovaletrate, α-copaene and bicyclogermacrene were in the Nevakoh seed oil, but not the Shahoo seed oil. Conversely, 3-carene, α-terpinene, 1,3,8-p-menthatriene, elemene (γ and δ), β -cubenone, β-cederene, γ-curcumene, δ-cadinene and spathulenol were found in Shahoo seed oil, but were not detected in the Nevakoh seed oil.

Table 1. A comparison of chemical composition of the essential oils from the seeds and leaves of *Ferulago angulata* collected at Nevakoh & Shahoo. Contents are given as a percent of the total oil. (T=trace) Nev.=Nevakoh samples, Sha.=Shahoo samples, RI_0= retention index *(-check this*)

Component	**RI**	**Nev. Leaf ٪**	**Sha., Leaf ٪**	**Nev. Seed ٪**	**Sha. Seed ٪**
α-Thujene	926	-	0.2	0.08	0.06
α-Pinene	937	27.1	25.7	15.4	7.29
Camphene	950	0.7	1.6	0.6	0.51
Dimethyl-bicyclo(3,1)hepta-2(8), 3- diene	952	0.4	-	-	-
Sabinene	970	1.5	2.1	0.4	.017
β-Pinene	977	1.3	1.7	0.8	0.23
Myrcene	983	5.2	2.0	1.9	1.05
α-Phellandrene	1003	0.3	0.2	0.6	0.72
3- Carene	1011	-	1.9	-	0.03
α - Terpinene	1013	-	-	-	0.02
Ortho-cymene	1016	0.2	-	-	-
p- Cymene	1018	-	-	4.1	1.4
Cis- ocimene	1031	22.6	27.9	64.8	76.11
Trans- ocimene	1040	3.3	1.3	1.4	2.26
γ- Terpinene	1053	0.2	0.1	5.9	2.88
α - Terpinolene	1063	-	-	0.3	0.59
Terpinolene	1083	0.2	0.1	-	-
Linalool	1085	1.5	-	-	-
1,3,8-p-Menthatriene	1112	-	0.1	-	0.03
Cis- epoxy ocimene	1114	-	-	0.1	-
Allo-ocimene	1120	0.3	1.6	0.6	2.38
Cis-verbenol	1131	1.1	-	0.2	-
Trans-verbenol	1135	5.8	-	0.2	0.26
p-mentha-1,5-dien-8-ol	1151	1.9	-	0.2	-
4 – Terpineol	1168	0.4	0.1	-	-
α- Terpineol	1178	1.2	0.2	-	-
Geraniol	1237	0.2	-	-	-
Tymol	1269	-	-	T	-
Bornyl acetate	1275	8.5	3.9	0.9	1.69
Myretenyl acetate	1285	0.6	-	T	-
Ipsdienol	1298	0.3	-	-	-
δ – Elemene	1342	-	-	-	0.02
Benzyl isovaletrate	1365	-	-	T	-
Methyl eugenol	1374	1.3	-	-	-
α - Copaene	1383	-	-	T	-
β-Cubenone	1384	0.3	1.0	-	0.05

Table 1. (Continued)

β - Bourbonene	1393	2.7	0.8	-	-
β - Cederene	1424	-	-	-	0.07
β - Caryophyllene	1427	0.2	0.9	-	-
Allo- aromadendrene	1449	-	0.1	-	-
α - Humulene	1459	-	0.2	-	-
γ – Curcumene	1478	-	-	-	0.34
γ- Muurolene	1480	-	.01	-	-
Germacrene D	1487	6.5	22.3	0.6	0.5
γ – Elemene	1500	-	1.1	-	0.25
Bicyclogermacrene	1501	1.3	-	0.2	-
Δ – cadinene	1523	-	0.4	-	0.16
Epigloubol	1531	0.6	-	-	-
Cadrol	1562	0.5	-	-	-
Spathulenol	1576	0.3	0.3	-	0.13
Caryophyllene oxide	1582	0.4	0.1	-	-
Aromadendrene oxide	1650	0.5	0.1	-	-
Neoclvenoxid–alkohol	1684	-	0.1	-	-

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