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Analysis by Gas Chromatography-mass Spectrometry of Essential Oil from Seeds and Aerial Parts of *Ferulago angulata* (Schlecht.) Boiss Gathered in Nevakoh and Shahoo, Zagross Mountain, West of Iran

Ghasempour Hamid Reza, Shirinpour Ebrahim and Heidari Hossien
Department of Biology, Faculatd of Science, University of Razi, Kermanshah, Iran 67149

Abstract: The essential oil from seeds and aerial parts of *Ferulago angulata* (schlecht.) Boiss growing in Nevakoh and Shahoo mountains, West of Iran was studied by gas chromatography and gas chromatography-mass spectrometry constituents were. The major components of seeds gathered in Nevakoh and shahoo were, respectively 24 and 26 constituents and found to be cis-ocimene (64.8 and 76.11%), α- pinene (15.4 and 7.29%), γ-terpinene (5.9 and 2.88%), ρ-cymene (4.1 and 1.4%), myrcene (1.9 and 1.05%) and bornyl acetate (0.9 and 1.69%). The major components of aerial parts gathered in Nevakoh and shahoo were respectively, 34 and 30 constituents and found to be α-pinene (27.1 and, 25.7%), cis-ocimene(22.6 and, 27.9%), bornyl acetate (8.5 and 3.9%), germacrene D (6.5 and 22.3%), trans-verbenol (5.8 and 0%), myrcene (5.2 and 2%), β-bourbonene (2.7 and 0%), p-mentah-1, 5-dien-8-ol (1.9 and 0%), sabinene (1.5 and 2.1%), linalool (1.5 and 0%), metyl eugenol (1.3 and 0%), bicyclogermacrene (1.3 and 0%) and α-terpineol (1.2 and 0%), 3-carene (0 and 1.9%), camphene (0 and 1.6%), γ-element (0 and 1.1%) and β-cubenone (0 and 1%).The major components which were common in both seeds and leaves and found to be, bornyl acetate, myrcene and trans-ocimene. The major components which were only found in aerial parts were germacreneD, β-bourbonene, camphene, sabinene and linalool. The major components which only were found in seeds: γ-terpinene and ρ-cymene.

Key words: *Ferulago angulata*, essential oil, α-pinene, cis-ocimene, trans-ocimene

INTRODUCTION

Ferulago belongs to the plant family Apiaceae (umbelliferae) subfamily Apioideae subtribe Ferulinae (Peucedaninae). The genus Ferulago comprises of thirty-five species world wide, of which seven species are found in Iran, consist of *F. stellata* Boiss, *F. bernardii* Tomk. and *M. pimen*, *F. phialocarpa* Rech. F., *F. subvelutina* Rech. F., *F. macrocarpa* (Fenzl) Boiss., *F. contracta* Boiss. and Hausskn. and *F. angulata* (Schlecht.) Boiss (Mozaffrian, 1983; Mozaffrian, 1996; Heywood, 1971).

F. angulata (Schlecht.) Boiss is distributed in east of Turkey, north of Iraq and Iran. The *F. angulata* have two subspecies, which includes: subsp. angulata (Schlecht) and subsp. Carduchorum (Boiss and Hausskn). The first subspecies is widespread in all mentioned countries but second subspecies is relic endemic of shahoo mountain, west of Iran (Rechhinger, 1987).

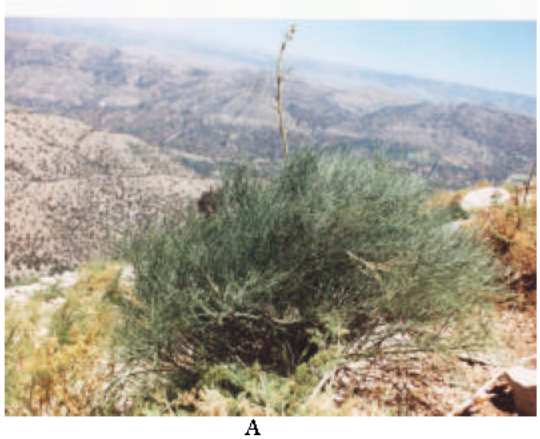
The *F. angulata* a perennial, dialypetal, yellow colour flower, compound umbel, compound thin narrow leaves and with the of height of 60-150 cm (Mozaffrian, 1983; Zargari, 1981). From the past, Man especially in Kordestan province has been using it as an additive to edible oil which has been made from farm animal's milk and also as a food preservative.

This species grows in altitude of 1900- 3200 m (above sea level) (measured by researcher's) (Rechinger, 1987). The essential oil components of *F. angulata* from two different collection sites were sampled, extracted and determined by GC and GC/Mass. The seeds and aerial parts were gathered both from Kermanshah state, Zagross mountain, west of Iran (In Kurdestan). Present search showed no other report on chemical analysis of *F. angulata* (Schlecht.) Boiss.

MATERIALS AND METHODS

Plant material: The air-dried aerial parts of *F. angulata* were gathered at May, 2004 and the seeds were collected at October, 2004 from two different mentioned ecotypes (Shahoo and Nevakoh mountains), kermashah province west of Iran. The sampling sites were at the altitudes of 2400-2600 m in Shahoo and 2000-2100 m in Nevakoh above sea level) (measured by researcher's) (Fig. 1).

Corresponding Author: Ghasempour Hamid Reza, Department of Biology, Faculatd of Science, University of Razi, Kermanshah, Iran 67149 Tel: 0098-831-4274545 Fax: 00988314274555



A

B

Fig. 1: A: *Ferulago angulata* plant; B: *Ferulago angulata* seed

Isolation procedure: One hundred gram aerial parts and 50 g seeds of *F. angulata* were powdered and placed in a flasks containing 1200 and 1000 mL of distilled water and steam distilled in a Clevenger-type apparatus according to British method for 3 h. The essential oil were dried over anhydrous Na_2SO_4 and stored at 4°C in the dark. Essential oil yield were 0.63% for leaves and 3.2% for seeds based on dried weight of the samples.

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

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 raised from 60 to 250°C at a rate of 5°C min^{-1} and then held at 250°C for 10 min.; transfer line temperature was 250°C. The quadrupole mass spectrometer was scanned over the 45-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA.

Identification of components: The constituents of the oil were identified by calculation of their retention indices under temperature-programmed conditions for *n-alkanes* (C_6-C_{24}) and the oil on a DB-1 and DB-Wax columns under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature (Shibamoto, 1987). Quantitative data was obtained from FID area percentages without the use of correction factors.

RESULTS

The component's comparison of the essential oil from the aerial parts and the seeds of *F. angulata,* their retention indices, their percentage composition and identification methods are given in Table 1 where the components are listed in order of elution on the DB-1 column.

The major constituents of the essential oil were found to be monoterpene hydrocarbons and sesquiterpene hydrocarbons. The major aerial parts components were found to be α-pinene (27.1 and 25.7%), cis-ocimene (22.6 and 27.9%), bornyl acetate (8.5 and 3.9%), germacreneD (6.5 and 22.3%), myrcene (5.2 and 2%), trans ocimine (3.3 and 1.3%), β-bourbonene (2.7 and 0.8%), camphene (0.7 and 1.6%), sabinene (1.5 and 2.1%), linalool (1.5 and 0%). Also, the major in seed constituents were as follow: Cis-Ocimene (64.8 and 76.11%), α-pinene (15.4 and 7.29%), γ-terpinene (5.9 and 2.88%), ρ-cymene (4.1 and 1.4%), trans-ocimene (1.4 and 2.26%) and bornyl acetate (0.9 and 1.69%).

Table 1: Percentage composition of the essential oils from the seeds and aerial parts of *Ferulago angulata*

Component	RI	A%	B%	C%	D%
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	0.17
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-Element	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
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	-	0.01	-	-
germacrene D	1487	6.5	22.3	0.6	0.5
γ-Element	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	-	-

A = Nevakoh's aerial parts sample, B = Shahoo's aerial parts sample, C = Nevakoh's seeds sample, D = Shahoo's seeds sample. (T = Trace)

CONCLUSION

In this research, major identified constituents of the extracts were: cis-ocimene, α-pinene, bornyl acetate, germacrene D, myrcene, trans-ocimene, β-bourbonene, camphene, sabinene, γ-terpinene, ρ- cymene and linalool.

Antibacterial effects of *alpha-terpineol* and *terpenen-4-ol* have been reported (Cosentino *et al*., 1999; Jedlickov'a, 1992). Also, some antifungal activity have been shown by *alpha-pinene*, *beta-pinene,* ρ- cymene and *linalool* constituents (Adam *et al*., 1998; Lis-Balchin *et al*., 1998). *In vitro* anti-lice activity of *Terpineol, alpha-pinene* and *camphene* have when tested (Yarnell, 1998). *Beta-pinene* may have antioxidant activity (Hohmann *et al*., 1999; Lopez-Arnaldos *et al*., 1994; Billany *et al*., 1995).

The major difference between the leaves components of two ecotypes were: myrcene, 3-carene, linalool, trans-verbenol, bornyl acetate, germacreneD. As measurements showed, ortho-cymene, linalool, Cis-Verbenol, trans-verbenol, cadrol, methyl euginol, ipsdienol, p-mentha-1,5-dien-8-ol and Bicyclogermacrene were the constituents which found only in Nevakoh province extract. Also, α-thujene, 3-Carene, α-humulene, allo-aromadendrene, 1,3,8-p-Menthatriene, Element (γ and δ), γ-muurolene were the constituents which found only in Shahoo province extract.

The major difference between the seeds components of two ecotypes were: cis-ocimene, α-pinene, ρ-cymene trans- ocimene and γ-terpinene. As measurements showed, Cis-epoxy ocimene, Cis-Verbenol, p-mentha-1,5-dien-8-ol, Thymol, Myretenyl acetate, Benzyl isovaletrate, α--Copaene and Bicyclogermacrene were the constituents which found only in Nevakoh province extract. Also, 3-Carene, α-Terpinene, 1,3,8-p-Menthatriene, Element (γ and δ), β-Cubenone, β-Cederene, γ-Curcumene, δ-Cadinene and Spathulenol were the constituents which found only in Shahoo province extract.

Soil analysis revealed that soil texture were the same in both ecotypes. Phosphorus amounts was higher in Nevakhoo ecotype. In contrast the amount of Fe and Mn were higher in Shahoo. In comparison, vegetation density was much more higher at Shahoo ecotype which might be due to higher availability of the mentioned micro elements. Also, the soil homos in Shahoo was higher than Nevakoh (with soil PH of 7.4 and 7.8, respectively).

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