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Chemical Composition of the Essential Oil of *Artemisia absinthium* Growing Wild in Iran

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Abstract: Studies were conducted to investigate the composition of essential oil of wormwood (*Artemisia absinthium* L.) growing wild in Iran. The wormwood aerial parts were harvested in full blooming time from an area between Deylaman and Asiabar villages, at Alborz altitudes in Guilan province in September 2005. Aerial parts were dried at shade (room temperature) for several days and their essential oil was extracted by hydrodistillation method in a Clevenger apparatus and analyzed by GC/MS. Results showed that essential oil yield was 1.3%. Twenty eight components representing 93.3% of the oil were identified, which were mostly monoterpenes. β- pinene and β- thujone were the main components, which their contents were 23.8 and 18.6% respectively. The largest part of the essential oil was formed by hydrocarbon monoterpenes (47.8%). The results proved that chemotype of the studied wormwood essential oil was specific and different from other wormwood essential oil chemotypes, which have been reported so far.

Key words: *Artemisia absinthium*, essential oil, β- pinene, β- thujone, Iran

INTRODUCTION

Artemisia absinthium L. is an aromatic plant of the family Asteraceae, subfamily Asteroideae, tribe Anthemideae and is known by the common names wormwood (UK), absinthe (France), wermut (Germany) and afsantine (Iran) (Zargari, 1989; Wright, 2002). The plant grows in North and East of Iran (Zargari, 1989) and also is very common in Turkey (Aslan *et al*., 2005; Kordali *et al*., 2006). The herb is native to warm Mediterranean countries, usually found growing in dry waste places such as roadsides, preferring a nitrogen-rich stony and hence loose soil. Wormwood has been naturalized in northeastern North America, North and West Asia and Africa. The plant's essential oil and bitter principles underlie its medicinal and commercial significance (Wright, 2002).

A. absinthium extracts and essential oils are used for healing various diseases (Lawless, 1999; Balz, 1996; Wright, 2002). Oil of *A. absinthium* has been found to repel fleas and flies (Duke, 1995) and mosquitoes (Morton, 1981) and to kill houseflies (Kaul *et al*., 1978). Anthelmintic, antibacterial, antifungal, insect repellent, narcotic, digestive, tonic and other bioactivities are characteristic of preparations from wormwood plants. Their stimulant property is dependent on bitter substances as artabsin (sesquiterpene lactone) and absinthin (dimmer of sesquiterpene lactones) present in plant extracts (Wright, 2002). Wormwood essential oil components 1, 8-cineole, cis (α) and trans (β)-thujones help people to withstand cold and other hardships of the Himalayan region (Wright, 2002; Aslan *et al*., 2005; Kordali *et al*., 2006). Large amounts of the above compounds are toxic (Woolf, 1999). Seizures may be caused by 1, 8- cineole and thujones for chronic users. In addition, thujones may evoke dementia (Wright, 2002).

The major constituent of wormwood oil is thujone, present at levels of approximately 40-70% of the oil (Tucker *et al*., 1993). Other constituents present at significant levels include myrcene (<35%), α- pinene (6%) and nerol (3%) in wormwood oil of Russian origin (Goraev *et al*., 1962); camphor (6%), ρ-cymene (4%), limonene (4%) and α- pinene (4%) in Spanish wormwood oil (Mugica and Ochoa, 1974); β-phellandrene (10%), α-humachalene (7%) and β- caryophyllene (5%) (Kaul *et al*., 1979; Aslan *et al*., 2005; Kordali *et al*., 2006). High levels of thujanol and thujyl acetate (60-70%), myrcene (35%), camphor and 1, 8-cineole were also determined in wormwood essential oils (Wright, 2002). Thujones, trans-sabinyl acetate, cis-chrysanthenyl acetate and cis-epoxyocimene are the most common constituents in wormwood essential oils (Arino *et al*., 1999; Wright, 2002; Juteau *et al*., 2003).

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This study was conducted to obtain information about essential oil of wormwood growing wild in Iran. It presents the chemical composition of essential oil from aerial parts of *A. absinthium* L. collected in a habitat from North of Iran.

MATERIALS AND METHODS

Plant material: The aerial parts of *A. absinthium* were collected in full blooming time from an area between Deylaman and Asiabar villages, at Alborz altitudes in Guilan province in September 2005. The aerial parts were dried at room temperature (20-25°C) for several days until measurement of weight. Voucher specimens were deposited in the Herbarium of the faculty of agricultural sciences, Shahed University, Tehran, Iran.

Isolation procedure: The essential oil was prepared by hydrodistillation for 2.5 h using a Clevenger-type apparatus. The oil was dried over anhydrous calcium chloride and stored in sealed vials at low temperature (2°C) before analysis.

Gas chromatography: GC analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m×0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 40°C for 5 min and then programmed to 250°C at a rate of 4°C min^{-1}. Injector and detector (FID) temperature were 260°C; helium was used as carrier gas with a linear velocity of 32 cm sec^{-1}.

Gas chromatography-mass spectrometry: GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (60 m×0.25 mm i.d.). Oven temperature was 40-250°C at a rate of 4°C min^{-1}, transfer line temperature 260°C, carrier gas helium with a linear velocity of 31.5 cm sec^{-1}, split ratio 1/60, ionization energy 70 eV, scan time 1 sec, mass range 40-300 amu.

Identification of components: The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds, or with data published in the literature (Adams, 1995; Shibamoto, 1987).

RESULTS AND DISCUSSION

The chemical composition of the *A. absinthium* essential oil is shown in Table 1. The components are listed in order of their elution on the DB-5 column.

Table 1: Chemical composition of the essential oil of *Artemisia absinthium*

Components	RI	(%)
α-pinene	938	3.8
sabinene	975	8.9
β-pinene	980	23.8
myrcene	990	4.0
α-phellandrene	1004	3.2
α-terpinene	1017	0.4
ρ-cymene	1025	1.9
β-phellandrene	1029	0.7
1,8-cineole	1033	0.3
(E)-β-ocimene	1048	0.4
γ-terpinene	1061	0.7
linalool	1097	4.2
α-thujone	1101	0.9
β-thujone	1113	18.6
iso-3-thujanol	1134	0.9
trans pinocarveol	1136	0.6
Terpinen-4-ol	1177	1.5
α-terpineol	1189	0.3
myrtenal	1193	0.3
germacrene D	1478	3.1
β-selinene	1488	0.7
α-dehydro-ar-himachalene	1514	3.8
γ-dehydro-ar-himachalene	1530	0.6
neryl isovalerate	1581	0.8
geranyl isovalerate	1604	1.9
Cubenol	1644	4.3
α-cadinol	1651	1.8
chamazulene	1730	0.9
Total		93.3
Monoterpene		75.4
Hydrocarbon monoterpenes		47.8
Oxygenated monoterpenes		27.6
Sabinene skeleton		29.3
Sesquiterpene		17.9
Hydrocarbon monoterpenes		9.1
Oxygenated sesquiterpenes		8.8
Oxygenated fraction		36.4
Hydrocarbon fraction		56.9

RI: Retention index on DB-5 column

Twenty eight compounds were identified in the essential oil of *A. absinthium*, representing 93.3% of the total oil components. The major components of the oil were found to be β- pinene and β- thujone, their contents being 23.8 and 18.6%, respectively. The other major components were sabinene, Cubenol, linalool, myrcene, α-pinene, α-dehydro- ar-himachalene, α-phellandrene and germacrene D. Monoterpenoids comprised a large part (75.4%) of wormwood oil. The largest part of the essential oil was formed by hydrocarbon monoterpenes (47.8%) and the other parts included oxygen-containing monoterpenes (27.6%), hydrocarbon sesquiterpenes (9.1%) and oxygen-containing sesquiterpenes (8.8%). Compounds with a sabinene carbon skeleton (sabinene, α and β- thujone, iso-3-thujanol) made up 29.3% of total oil.

The oil isolated by hydrodistillation from the aerial parts of *A. absinthium* was found to be dark green to brown liquid that can be attributed to the presence of chamazulene and its yield was 1.30% (v/w). Essential oil contents between 0.2 and 1.5% in the crude drug have been reported in the literature (Wichtl, 1989). The most abundant constituent in the essential oil was β-pinene, which has not been reported for the species from other countries till now. It can be said that β-pinene is a new component for wormwood oil and possibly restricted to Iran. It has been reported that *A. absinthium* plants produce several chemotypes of essential oils in different countries and the main component of wormwood essential oils is thujone (Juteau *et al.*, 2003; Wright, 2002). A sample collected in Argentina, had the β-thujone (60%) as main constituent (Sacco and Chialva, 1988). The plants and their parts from Croatia contained notable amounts of (Z)-6, 7-epoxyocimene besides the first major constituent trans-thujone (Juteau *et al.*, 2003). Some volatile oils from North Italy (Wright, 2002), France (Juteau *et al.*, 2003; Wright, 2002) and Spain (Arino *et al.*, 1999) did not contain thujones. The predominant components of the above oils were (Z)-6, 7-epoxyocimene and/or (Z)-chrysanthenyl acetate. Two chemotypes were determined in Spain: one oil contained both the above compounds as the main ones, but another oil was characterized by (Z)-6, 7-epoxyocimene with minor amounts of other constituents (Arino *et al.*, 1999). Carnat *et al.* (1992) found cis-chrysanthenol as the major component in the oil of plants grown in central France. Sabinyl acetate prevailed in some oils from different countries (Arino *et al.*, 1999; Wright, 2002). Chiasson *et al.* (2001) reported β-thujone as a main component (32.1%) in wormwood essential oil from Canada. Oils isolated from native and cultivated wormwood plants growing in Oregon contained β-thujone (40%) and cis-epoxyocimene (25%) in the native plants and sabinene (30%) and myrcene (30%) in the cultivated plants (Tucker *et al.*, 1993). Bornyl acetate was reported as the major constituent (23%) of a sample collected in Cuba (Pino *et al.*, 1997), but it was not observed in the present study.

It is suggested that the studied wormwood in this work synthesizes a kind of essential oil component, which is different from other components reported so far. The quality and yield of essential oils from *Anthemideae* plants are influenced by the various factors such as harvesting season (Cornu *et al.*, 2001), fertilizer and the pH (ideal in acidic, pH 4.5-5.4) of soils (Alvarez-Castellanos and Pascual-Villalobos, 2003), the choice and stage of drying conditions (Tateo and Riva, 1991), the geographic location (Maffei *et al.*, 1994), subspecies (Goren *et al.*, 2001), choice of plant part or genotype (Mishra *et al.*, 1999; Nori-Shargh *et al.*, 1999; Keskitalo *et al.*, 2001) or extraction method (Scalia *et al.*, 1999).

Monoterpenoids comprised a large part of wormwood oil in the study. This corroborates the finding of Judpentienë and Mockutë (2004) which reported that monoterpenoids comprise a large part (55.7-80.2%) of wormwood oil in Lithuania. The largest part of the essential oil was formed by hydrocarbon monoterpenes (47.8%) and the other parts included oxygen-containing monoterpenes (27.6%), hydrocarbon sesquiterpenes (9.1%) and oxygen-containing sesquiterpenes (8.8%). Judpentienë and Mockutë (2004) reported that the largest part of the essential oil in wormwood is formed by oxygenated monoterpenes (47.1-66.7%) and the hydrocarbon monoterpenes were 8.2-24%.

Compounds with a sabinene carbon skeleton made up 29.3% of total oil. These compounds were comprised (33.9-61.0%) of wormwood oil in Lithuania (Judpentienë and Mockutë, 2004). Chamazulene (0.9%) was present in the oil which contributed to the characteristic colour of wormwood oil. A small percentage of chamazulene (0.2%) was found in the Cuban oil (Pino *et al.*, 1997) and in two chemotypes studied of Spanish oil it was 3 and 0.3% (Arino *et al.*, 1999).

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