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Variations in Essential Oil Components in Cultivated and Regenrated *Artemisia Absinthium* L.

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The chemical composition of the essential oil of *Artemisia absinthium* L., grown under *in vitro*, greenhouse and field conditions was examined by GC and GC-MS. Significant quantitative and qualitative differences in the composition of the oils were observed. α-Thujone was the main constituent of the greenhouse and field grown samples while it was absent in the oil of the regenerated plantlets.

Key Words: ***Artemisia absinthium* L., Asteraceae, Essential oil composition, α-Thujone.**

INTRODUCTION

Artemisia absinthium L. belongs to the family Asteraceae, consisiting of more than 800 species that are widespread all around the world. This genus is represented by 31 species which grow wild in Iran. In this genus, many species have been used as folk remedies with a large number of medicinal uses, including antimalarial, antiviral, antitumor, spasmolytic, etc.[1] *Artemisia absinthium* L. (wormwood), a perennial aromatic plant, with the common Persian name Afsantin, is included in the subgenus *Artemisia*[2].

EXPERIMENTAL

This study was based on plants generated from seeds of *Artemisia absinthium* L. originally obtained from Hamadan Medicinal Plants Garden. Shoots obtained from 2 years old field grown plants derived from single selected clone were used as the explant source. Shoot tips were surface disinfected with sodium hypochlorite (0.7%) for 10 min and rinsed several times with sterile distilled water. Explants were cultured in 100 mL Erlenmeyer flasks containing 25 mL of Murashige and Skoogs (MS) basal medium supplemented with α-naphthalenacetic acid (NAA) (0.5 mg/L), 6-benzyladenine pourine (BAP) (0.2 mg/L) and sucrose (20 g L^{-1}). The pH of medium was adjusted to 5.7 with NaOH 1 N and Difco-bacto agar added at

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7 g L^{-1} before autoclaving for 20 min at 121°C and 108 kPa. The newly formed shoots were multiplied by subculturing every 30 days. *In vitro* plants were about 7 cm in height when analyzed.

Seedlings obtained from *in vitro* culture were transferred into a greenhouse to 15 cm pots filled with a mixture of 20% top soil, 40% peat moss (sphagnum) and 40% perlite under 16 h photoperiod. Seedlings were maintained at 27°C (day)/ 25°C (night). Field plantings were made with transplants grown at the greenhouse. Soil type was silt loam. Field plants were more robust and had more vegetative mass than greenhouse plants. Greenhouse and field plants were about 35 and 70 cm in height when analyzed.

Isolation procedure: The volatile oils were obtained by hydrodistillation of the dry aerial parts of *A. absinthium*, grown under *in vitro*, greenhouse and field conditions, on a Clevenger-type apparatus, which gave the essential oil in 0.24, 0.63 and 0.81% yield (w/w), respectively.

The sample oil was dried over anhydrous sodium sulfate and stored in sealed vials at low temperature before analysis.

GC: GC analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-1 fused silica column (60 × 0.25 mm, 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; injector and detector (FID) temperature was 260°C; carrier gas helium with a linear velocity of 32 cm/s. Quantitative data was obtained from FID area percentage without the use of correction factors.

GC-MS: GC-MS analysis was carried out on a Varian 3400 GC-MS system equipped with a DB-1 fused silica column (60 × 0.25 mm, film thickness 0.25 μm). Oven temperature 50–250°C at a rate of 4°C/min, transfer line temperature 260°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, ionization energy 70 eV, scan time 1 s, mass range 40–300 amu.

Identification of components: The components of the oils 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 samples or with data published in the literature[3, 4].

RESULTS AND DISCUSSION

The oil isolated by hydrodistillation from aerial parts of *A. absinthium* grown under *in vitro*, greenhouse and field conditions was obtained in yields of 0.24, 0.63 and 0.81% (w/w), respectively. The compounds identified with their percentage are given in Table-1 and also the compounds are listed in order of their elution from DB-1 column.

Comparison of the results revealed that volatile monoterpenes were absolutely absent in the oil of regenerated plantlets. In this case, the oil was rich in citronelyl isovalerate (22%) and terpinyl isobutyrate (11%), where both compounds were not detected in the other samples.

Occurrence of chamazulene in the oil of *A. absinthium* was reported in the range of 0–4% according to the habitat conditions of the plant[5, 6]. In the present study, chamazulene was only found in the regenerated plantlets.

TABLE-1
PERCENTAGE COMPOSITION OF THE OIL FROM *ARTEMISIA ABSINTHIUM* L. GROWN UNDER DIFFERENT CONDITIONS

Compound	RI	*In vitro* (%)	Greenhouse (%)	Field (%)	Method of identification
α-pinene	942	—	7.4	18	RI, MS, Co-I
Sabinene	972	—	1.4	3.0	RI, MS, Co-I
Myrcene	985	—	0.7	1.5	RI, MS
Limonene	1021	—	—	0.5	RI, MS, Co-I
cis-Ocimene	1026	—	—	0.6	RI, MS
α-Pinene oxide	1072	—	—	0.3	RI, MS
Terpinolene	1077	—	—	0.5	RI, MS
E-Pineone oxide	1089	—	—	0.3	RI, MS
α-Thujone	1108	—	41	60	RI, MS
β-Thujone	1117	—	3.0	5.5	RI, MS
Borneol	1148	10.5	0.6	0.2	RI, MS, Co-I
cis-Limonenoxide	1160	—	—	0.8	RI, MS
Bornyl formate	1205	—	—	0.3	RI, MS
Bornyl acetate	1264	1.5	4.7	1.7	RI, MS, Co-I
Terpinenyl acetate	1318	3.6	—	—	RI, MS
Neryl acetate	1336	2.0	—	—	RI, MS
Geranyl acetate	1355	—	2.7	—	RI, MS
α-Copaene	1372	—	27.5	—	RI, MS
E-Cedrene	1410	—	—	0.6	RI, MS
E-Caryophyllene	1414	0.5	1.8	—	RI, MS
α-Humulene	1439	—	3.7	—	RI, MS
Linalyl isopentanoate	1461	3.5	—	—	RI, MS
Terpinyl isobutyrate	1464	11	—	—	RI, MS
γ-muurolene	1472	—	—	0.3	RI, MS
Engenyl acetate	1484	9	—	—	RI, MS
Calamene	1518	—	1.0	—	RI, MS
trans-Nerolidol	1550	12	—	—	RI, MS
Unknown	1554	11.5	—	—	RI, MS
Citronelyl isovalerate	1557	22	—	—	RI, MS
Spathulenol	1566	1.0	—	—	RI, MS
Caryophyllene oxide	1576	2.5	—	0.5	RI, MS
Cedrol	1585	0.4	—	—	RI, MS
Globulol	1588	—	4.0	5.0	RI, MS
α-Cadinol	1639	1.0	—	0.3	RI, MS
α-Eudesmol	1643	—	0.5	—	RI, MS
Chamazulene	1702	0.5	—	—	RI, MS

RI = Retention index, MS = Mass spectrum, Co-I = Coinjection with an authentic sample.

α-Thujone was the main component of the greenhouse and field grown plants (41 and 60%, respectively). This compound was not produced in the regenerated plantlets. α-Pinene, β-thujone, bornyl acetate and globulol were also found in considerable amounts in both samples. An interesting point was the presence of 27.5% of α-copaene in the oil of greenhouse plant that was not observed in other samples.

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