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Chemical composition and biological effects of essential oils from *Artemisia absinthium* L. cultivated under different environmental conditions

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ABSTRACT

The objective of this study was the valorization of the essential oils from Spanish *Artemisia absinthium* domesticated plants from Teruel and Sierra Nevada (Spain). These populations were experimentally cultivated in the field and under controlled conditions. The insect antifeedant properties of their essential oils collected yearly from two locations were tested against *Spodoptera littoralis*, *Myzus persicae* and *Rhopalosiphum padi*. Additionally we studied their phytotoxic, antifungal and antiparasitic effects. The oils from cultivated *A. absinthium* were characterized by the presence of *cis*-epoxyocimene, chrysanthenol, and chrysanthenyl acetate. The variations observed in oil composition were mostly quantitative but also qualitative. (Z)-2,6-Dimethyl-5,7-octadien-2,3-diol has been isolated and identified by NMR. Among the oil samples, these rich in *cis*-epoxyocimene and sesquiterpenes were the most active ones against *S. littoralis*. (Z)-2,6-Dimethyl-5,7-octadien-2,3-diol showed moderate activity against *S. littoralis*. The strongest antifeedant effects were found for commercial *A. absinthium* oil samples rich in thujones and sabinyl acetate. *F. oxysporum* and *F. solani* were affected by oils from cultivated *A. absinthium* and commercial oil samples. Oils from cultivated *A. absinthium* showed antiparasitic effects against *Leishmania infantum* and *Trypanosoma cruzi* with better results than the commercial samples.

1. Introduction

The genus *Artemisia* is a genus that belongs to the family Compositae (Asteraceae) and consists of about 500 species distributed through the world (Bora and Sharma, 2011). *Artemisia absinthium* L. is an aromatic and medicinal plant of ethnopharmacological interest (Bora and Sharma, 2010; Lachenmeier, 2010). The composition and biological effects of the essential oil (EO) of *A. absinthium* has been widely studied. *A. absinthium* EOs had antimicrobial (Baykan Erel et al., 2012; Gandomi Nasrabadi et al., 2012; Juteau et al., 2003) and antiprotozoal effects against *Leishmania aethiopica* and *L. donovani* (Tariku et al., 2011). In addition, thujone-rich oils have been shown to have acaricidal (Chiasson et al., 2001), insecticidal (Kaul et al., 1978; Kordali et al., 2006; Umpiérrez et al., 2012) and fungicidal effects (Umpiérrez et al., 2012) and myrtenol-rich oils repelled fleas, flies, mosquitoes (Erichsen-Brown, 1979) and ticks (Jaenson et al., 2005).

Among the major EO components reported are α and β-thujone (Carnat et al., 1992; Chialva et al., 1983; Geszprych et al., 2010; Umpiérrez et al., 2012), myrcene, trans-sabinyl acetate (Geszprych et al., 2010; Judzentiene et al., 2012; Karp and Croteau, 1982; Lopes-Lutz et al., 2008; Sharopov et al., 2012), β-pinene, (Kordali et al., 2005), 1,8-cineole (Kordali et al., 2005; Tehrani et al., 2012), camphor (Tariku et al., 2011), *cis*-epoxyocimene (Chialva et al., 1976), chrysanthenil acetate (Chialva et al., 1983; Geszprych et al., 2010; Kordali et al., 2005), sabinene (Baykan Erel et al., 2012; Geszprych et al., 2010; Kordali et al., 2005), myrtenol (Erichsen-Brown, 1979; Jaenson et al., 2005), bornyl acetate (Pino et al., 1997), artemisia ketone, linalool, hydrocarbon monoterpenes (Kordali et al., 2005), sesquiterpene lactones (Leung, 1980; Martín et al., 2011a) and mixtures of some of these components (Carnat et al., 1992; Chialva et al., 1983; Geszprych et al., 2010), depending on the plant origin.

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A. absinthium is abundant in the mountains of Spain as ruderal species and it is used as a medicinal remedy. There are seven chemotypes described in the Iberian Peninsula and some of them are thujone-free (Ariño et al., 1999). Therefore, the use of *A. absinthium* based on the collection of wild populations can result in variable compositions of the extracts. As part of an ongoing project on the valorization of native plants and the sustainable production of botanical biopesticides, Spanish populations of wormwood have been domesticated for experimental cultivation in the field and under controlled conditions (Burillo, 2009; Gonzalez-Coloma et al., 2012; Martín et al., 2011a, 2012) to generate agronomic (Burillo, 2009) and chemical data. The biological effects (insect antifeedant action and antioxidant effect) and constituents of the ethanolic extracts (OSE) of these two populations have been described as a function of cultivation method, location and time, with the sesquiterpene lactone hydroxypelenolide being the major component followed by the flavones artemetin, and casticin. Casticin concentrations correlated with the antifeedant and antioxidant effects of these wormwood extracts. Furthermore, optimized supercritical fluid extracts of cultivated Spanish *A. absinthium* showed an improvement in the yield of several mono- and sesquiterpenes and were more active than the traditional extracts (EO, OSE) against insects (Martín et al., 2011a, 2012).

Here we report on the chemical composition of the essential oils of domesticated *A. absinthium* plant samples collected yearly from two locations and from clonic plants cultivated under controlled conditions. One unidentified major component was isolated and identified by NMR experiments as the monoterpene (Z)-2,6-dimethylocta-5,7-dien-2,3-diol. The insect antifeedant properties (*Spodoptera littoralis, Myzus persicae and Rhopalosiphum padi*), antifungal (*Fusarium* spp.) and phytotoxic (*Lactuca sativa, Lolium perenne*) effects of the EOs were tested. Additionally we studied their antiparasitic effects on *Trypanosoma cruzi* and *Leishmania infantum*.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were determined in $CHCl_3$ at room temperature using a Perkin-Elmer 137 polarimeter. IR spectra were taken on a Perkin-Elmer 1600 FT spectrometer. UV spectra were measured on a Hewlett-Packard HP-8254-A. NMR spectra were measured on a Bruker AMX2 500 MHz spectrometer with pulsed field gradient using the solvent as internal standard ($CDCl_3$, at δ_H 7.26 and δ_C 77.0). The programs used in two-dimensional (2D) NMR experiments (HMBC, HSQC, COSY, and NOESY) were those furnished with the manufacturer's software. EIMS and exact mass measurements were recorded on a Micromass Autospec instrument at 70 eV. Preparative and semipreparative HPLC was carried out with a Beckman Coulter 125P equipped with a diode-array detector 168 and preparative Interstil silica 20 mm × 250 mm, 10 μm particle size and semipreparative Ultrasphere silica 10 mm × 250 mm, 5 μm particle size columns. Silica gel 60 F_{254} (Merck, art. 105715) and Sephadex LH-20 (Pharmacia) were used for column chromatography. Compounds were visualized on TLC with Oleum reagent.

2.2. Plant material and cultivation

Plant material for field cultivation was selected from a wild population growing in Teruel (Spain). The individuals for field cultivation were obtained from seeds. The experimental fields were located in Barrio de San Blas, Teruel, Spain (T) and Ejea de los Caballeros, Zaragoza, Spain (E). A detailed description of these fields and the cultivation parameters has been published (Burillo, 2009).

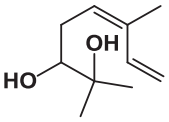

Fig. 1. (Z)-2,6-dimethylocta-5,7-dien-2,3-diol (**12**).

Flowering plant samples from 30 randomly selected plants were collected yearly and processed for EO extraction. The EOs analyzed corresponded to crops collected for 5 consecutive years (E1-E5 and T2-T5, Table 1).

The individuals for growth chamber, aeroponic orgreenhouse cultivation (SN) were obtained from a population from the nursery at the Sierra Nevada National Park (Granada) and cultivated as described for one year (Gonzalez-Coloma et al., 2012). Aerial parts of the growth chamber, green house and aerponically grown plants were collected for extraction (SNC, SNI and SNA samples respectively). For comparison purposes, a wild population V (Villacampa, Huesca, Aragón, 2006) and commercial *A. absinhium* EO samples H of different geographical origin (H1, commercial essential oil; H1.2, waste oil fraction rich in thujones and terpenes and H1.3, commercial oil fraction for the agrifood industry, rich in sesquiterpenes, esters and azulenes; Hausmann Aromatic S.A., San Andrés de la Barca, Barcelona, Spain) were also included in the study. A summary of all the samples studied is shown in Table 1.

2.3. Essential oil extraction and analysis

Plant samples (100 g) were distilled in a Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia (7th Ed., 2010). The essential oils were analyzed by GC-MS using an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, California, USA) coupled to an Agilent 5973N mass detector (electron ionization, 70 eV) (Agilent Technologies, Palo Alto, California, USA) and equipped with a 25 m × 0.20 mm i.d. capillary column (0.2 μm film thickness) HP-1 (methyl silicone bonded) (Hewlett-Packard). Working conditions were as follows: split ratio (30:1), injector temperature, 260 °C; temperature of the transfer line connected to the mass spectrometer, 280 °C; column temperature 70 °C for 5 min, then heated to 270 °C at 4 °C min^{-1}. EI mass spectra and retention data were used to assess the identity of compounds by comparing them with those of standards or found in the Wiley Mass Spectral Database (2001). Quantitative data were obtained from the TIC peak areas without the use of response factors.

2.4. Compound isolation

A bulk acetone extract (260 g, 25.8% yield) of the E2 sample (1000 g plant dry weight, 48.9% water content) was chromatographed on a vacuum liquid column (VLC) and eluted with n-hexane:EtOAc:MeOH gradients to give 8 fractions. Fraction 3 (3.9 g, 0.39%), was further chromatographed on Sephadex LH20, Si-gel column and preparative HPLC to give the monoterpene (Z)-2,6-dimethylocta-5,7-dien-2,3-diol (25.2 mg, 2.5×10^{-3}%).

^{1}H-NMR, MS and ^{13}C-NMR spectra of (Z)-2,6-dimethylocta-5,7-dien-2,3-diol coincided with those reported by Tsankova and Bohlmann (1983) (Fig. 1).

2.5. Insect bioassays

S. littoralis M. persicae and *R. padi* colonies were reared on artificial diet, bell pepper (*Capsicum annuum*) and barley (*Hordeum vulgare*) plants, respectively, and maintained at 22 ± 1 °C, >70% relative humidity with a photoperiod of 16:8 h (L:D) in a growth chamber. The bioassays were conducted with newly emerged *S.*