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Effects of abiotic environmental conditions on amount and enantiomeric composition of α-pinene in *Juniperus communis* L.

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ABSTRACT

Effects of some abiotic environmental conditions on amounts of essential oil and α-pinene therewith and enantiomeric composition of α-pinene in *Juniperus communis* L. were studied. The leaf and unripe cone samples of total 110 cone-bearing *J. communis* individuals were sampled from 11 natural habitats, differing mainly by illumination intensity and soil chemical characteristics, across Lithuania. The analysis of α-pinene in essential oils of leaves and unripe cones was carried out by GC-FID, and the enantiomeric analysis of α-pinene – by chiral-phase capillary GC. The study confirmed that the unripe cones accumulate more essential oils than leaves (1.3 ± SD 0.63% and 0.4 ± SD 0.14%, respectively), and the amount of essential oils in leaves negatively significantly correlated with illumination ($r = -0.69$, $p < 0.05$) and soil acidity ($r = -0.83$, $p < 0.05$) of habitats. The α-pinene was the main essential oil compound in 97% and 98% of all analysed leaf and unripe cone samples, respectively. Leaves and unripe cones contained very similar amounts of this monoterpene (54.1 ± SD 13.9% and 58.0 ± SD 14.62%, respectively). Significant positive correlations between the percentage of α-pinene and the ratio of (1R)-(+)-α-pinene/(1S)-(−)-α-pinene in essential oils of cones and leaves implies that the synthesis of this monoterpene and its enantiomers both in leaves and cones is interdependent. It was established that the (1R)-(+)-α-pinene is more prevalent than (1S)-(−)-α-pinene both in leaves and unripe cones of *J. communis* growing wild in Lithuania, and that purity of (−) enantiomer varied more than purity of (+) enantiomer. Our study showed that the amount of α-pinene and its enantiomeric composition did not correlate significantly with the illumination and soil characteristics of habitats neither in leaves nor in unripe cones.

1. Introduction

Juniperus communis L., or common juniper, is a woody plant accumulating essential oils which are recognized by the official pharmacopoeias of many countries (Pauli and Schilcher, 2004), as well as European Pharmacopoeia (European Pharmacopoeia, 2008). The species contains up to 2.3% of essential oils in unripe cones, up to 1.1% in ripe cones and up to 0.4% in leaves (Butkienė et al., 2006). The medicinal properties of *J. communis* depend mostly on monoterpene α-pinene (Cavaleiro et al., 2006; Leite et al., 2007), the contents of which vary greatly reaching 80.4% of the total essential oil content (Angioni et al., 2003; Shahmir et al., 2003; Butkienė et al., 2006, 2009; Filipowicz et al., 2009). The enantiomerisation is characteristic of α-pinene, which (1R)-(+) and (1S)-(−) enantiomers may exhibit quite different biological activity (Lis-Balchin et al., 1999; Filipowicz et al., 2003). The yield and composition of essential oils, the ratio of enantiomers of terpenes, as well as α-pinene, vary between different plant parts of *J. communis* (Butkienė et al., 2006; Hener et al., 1990;

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Sybilska et al., 1994; Ochocka et al., 1997). It is common that environmental factors influence the quantitative and qualitative composition of the essential oils of *J. communis*. However, the reasons of differences in enantiomeric composition of terpenes in the essential oils of junipers are still unknown.

J. communis is the species of a global distribution exhibiting wide range of ecological adaptation (Navasaitis, 2004). In Lithuania it grows as scattered by single individuals as well as in stands. However, few pure stands have left with areas exceeding 1.0 ha, while there are much more pinewoods and mixed forests where the species is among the major components of the undergrowth. In Lithuania *J. communis* occurs mostly in dry pinewoods, mixed forests, on river slopes, being light demanding, but also shade tolerant. Therefore the main objective of the current study was to establish the effects of some abiotic environmental conditions on amounts of essential oil and α-pinene therewith and composition of (1R)-(+) and (1S)-(−) enantiomers of α-pinene in unripe cones and leaves of *J. communis*, growing wild in Lithuania.

2. Materials and methods

2.1. Plant material

The leaves and unripe cones of *J. communis* were sampled from 11 natural habitats which differed by illumination intensity and soil characteristics, such as soil acidity, contents of organic nitrogen, mobile phosphorus, mobile potassium and humus, across Lithuania in August 2010 (Table 1). The unripe cones were taken for the present study as it was observed that the ratio of R and S enantiomers of α-pinene is the same both in ripe and unripe cones of *J. communis* (Ložienė et al., 2010). Ten individuals were randomly selected in each habitat. The plant material was collected separately from each of the individuals and dried at room temperature.

2.2. Evaluation of illumination and soil characteristics

The illumination was measured in kiloluxes with photoradiometer HD 2302.0 in three places per habitat at around the midday time and a clear sky condition.

Soil samples were taken from the depth of 10–15 cm at three randomly chosen, remote from each other places per habitat. Soil acidity (pH) was estimated electrometrically using 1 M KCl solution. The contents of organic nitrogen (N_2), mobile phosphorus (P_2O_5) and humus in the soil were estimated photoelectrocolorimetrically, and mobile potassium (K_2O) – by flame photometry.

2.3. Isolation of essential oil

The essential oils of leaves and cones from 110 samples (=trees) each were isolated by hydrodistillation in European Pharmacopoeia apparatus during 2 h.

2.4. Analysis of α-pinene by GC-FID and α-pinene enantiomers by chiral-phase capillary GC

1% essential oils solutions were prepared in mixture of diethyl ether and *n*-pentane (1:1) for further investigations. The analysis of α-pinene and its enantiomers were carried out by a FOCUS GC (Thermo Scientific) gas chromatograph with a flame ionisation detector (FID). Data were processed with the CHROM-CARD S/W.

The silica capillary column TR-5 (30 m, i.d. 0.25 mm, film thickness 0.25 μm) was used for the analysis of the monoterpene α-pinene with the following GC parameters: carrier gas helium flow rate 1.6 ml/min; temperature programme from 40 °C to 250 °C increasing at 4 °C/min; detector temperature 260 °C; split injector was heated at 250 °C. The identification of α-pinene was carried out by the comparison of the retention time (RT) of its GC peaks in the FID chromatograms with the RT of α-pinene analytical standard (Sigma–Aldrich). The percentage amounts of α-pinene were recalculated according to the areas of the FID chromatographic peaks assuming that all constituents of the essential oil comprise 100%.

α-pinene enantiomers were separated on HP–Chiral–20B column (30 m length, 0.249 mm id, 0.25 μm film thickness) with helium as carrier gas. The following GC parameters were used for the analysis of the α-pinene enantiomers: helium flow rate of 1.6 ml/min; temperature program from 85 to 160 °C increasing at 5 °C/min; detector temperature 260 °C; split injector was heated at 250 °C. The identification of (1R)-(+)-α-pinene and (1S)-(−)-α-pinene was carried out by the comparison of the retention time (RT) of its GC peak in FID chromatograms with the RT of (1R)-(+)-α-pinene and (1S)-(−)-α-pinene analytical terpene standard (Sigma–Aldrich; purity (GC area %) ≥98.5% and ≥99.0%, respectively) under the same GC parameters and column. The percentage amounts of α-pinene enantiomers were recalculated according to the areas of the FID chromatographic peaks assuming that monoterpene α-pinene fraction is 100%.

2.5. Statistical analysis

Statistical data processing (the calculation of means, standard deviations (SD), coefficients of variation (CV), Pearson's correlation coefficients (r), Fisher's criteria (F) and their reliabilities (p), one way analysis of variance (ANOVA)) was carried out with the STATISTICA 7 and MS Excel 2010.