Nature Research Centre, Institute of Botany, Vilnius, Lithuania

VARIATION OF α-PINENE AMOUNT IN ESSENTIAL OILS OF *JUNIPERUS COMMUNIS* L. GROWING WILD IN LITHUANIA

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Juniperus communis L., or common juniper, is the species of a global distribution exhibiting wide range of ecological adaptation.

In Lithuania *Juniperus communis* grows as separately as well as forms stands.

However, few pure stands have left with areas exceeding 1.0 ha, while there much more pinewoods and mixed forests where the species is among the major components of the undergrowth.



Juniperus communis occurs in Lithuania in dry pinewoods, mixed forests, on river slopes, being light demanding as well as shade tolerant.



Juniperus communis accumulate essential oils which are recognized by the official pharmacopoeias of many countries as well as European Pharmacopoeia.

Juniperus communis 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 *Juniperus communis* depend mostly on monoterpene α-pinene (Cavaleiro, 2006; Leite et al., 2007), the contents of which vary greatly reaching 80.4 % of the total essential oil content (Angioni et al., 2003; Shamir et al., 2003; Butkienė et al., 2005, 2006).

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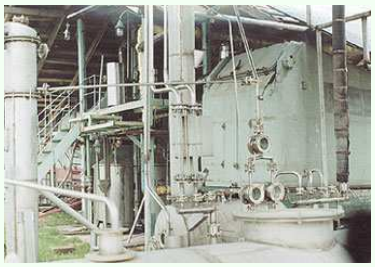
The private enterprise UAB "Mėta"



Method of extraction:
Juniper essential oil is steam-distilled from the berries alone or from the berries, needles and twigs of the shrub.

Properties:
general stimulant; against rheumatism; diuretic, antiseptic; against colds, expectorant, cough relief.

Usage and doses:

For Baths: 6-7 drops add to glass of yogurt or milk and pour in to bath.
Massage: add 3-4 drops of essential oil to 20g of basic oil.
Vaporizers: 1-2 drops for 5 m2 of room.
Compresses: 3-4 drops of non-diluted essential oil instill on to dry soft fabric.

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Our previous work* showed that both unripe and ripe cones of *Juniperus communis* synthesize similar amounts of α-pinene.

similar amounts of α-pinene

Therefore in this study the unripe cones were investigated.

*Ložienė K., Labokas J., Venskutonis P. R., Maždžierienė R. (2010): Chromatographic evaluation of the composition of essential oil and α-pinene enantiomers in *Juniperus communis* L. berries during ripening. – Journal of Essential Oil Research, 22: 453–458.

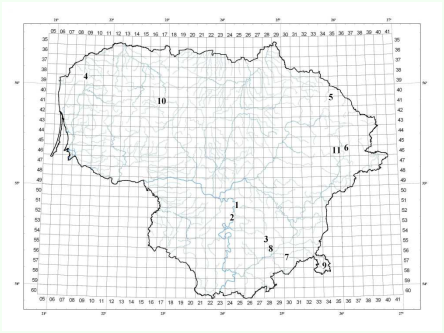
The main objectives of the study were:

-to measure the variation of percentage amounts of essential oil and α-pinene in unripe cones of *Juniperus communis,* growing wild in Lithuania, and in leaves as the main biomass producer,

-to establish the effects of some abiotic environmental conditions on amounts of essential oil and α-pinene in unripe cones and leaves of *Juniperus communis*.

Materials (1): sampling of plant material

leaves and unripe cones of *Juniperus communis* sampled from 11 different habitats across Lithuania



10 cone-bearing individuals per habitat selected for the sampling of leaves and unripe cones

in total 110 leaves and 110 unripe cones samples collected in August 2010

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Materials (2): habitats characterisation

11 investigated habitats differed by light and some soil characteristics (the soil acidity and the contents of organic nitrogen, mobile phosphorus, mobile potassium and percent humus)

Habitat No.	Characteristics of habitats					
	Light (klux)	N_2 (%)	P_2O_5 (mg/kg)	K_2O (mg/kg)	Humus	pH_{KCL}
1	35	0.20±0.03	67±23	156±21	3.6±0.4	6.5±0.6
2	31	0.33±0.06	153±35	311±72	4.9±0.3	7.3±0.1
3	28	0.31±0.08	180±72	194±106	4.9±1.5	5.1±0.4
4	30	0.25±0.11	54±41	78±18	5.1±1.8	5.1±0.9
5	30	0.18±0.03	86±26	131±10	3.3±0.2	6.7±0.3
6	29	1.01±0.30	75±14	71±13	14.4±4.6	7.7±0.1
7	12	0.05±0.00	88±16	16±3	1.7±0.2	4.3±0.1
8	11.5	0.13±0.03	149±51	25±6	3.7±0.5	4.2±0.0
9	5	0.04±0.01	103±7	24±1	1.4±0.2	5.6±0.2
10	13	0.07±0.02	115±6	28±10	2.1±0.9	4.1±0.2
11	9	0.04±0.02	88±16	24±4	1.9±0.4	4.8±0.6

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Methods (1): essential oils isolation and analysis

pure essential oils of leaves and cones from 110 samples (=trees) each isolated by hydrodistillation in a European Pharmacopoeia apparatus during two hours

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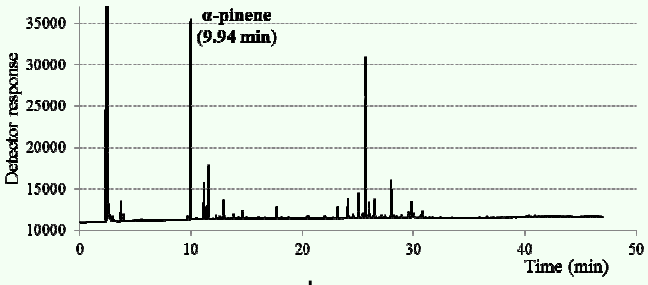
1% essential oils solution prepared in diethyl ether : pentane (1:1)

↓

1% essential oils solutions analysed on GC-FID by silica capillary column TR-5
(30 m, i.d. 0.25 mm, film thickness 0.25 µm);
carrier gas - helium (1.6 ml/min);
detector temperature - 260°C;
oven temperature - from 40°C to 250°C at the rate of 4°C/min;
split injector - 250°C;
split ratio - 15:1

Methods (2): α-pinene analysis

identification of α-pinene carried out by the comparison of retention time (RT) of its GC peaks in FID chromatograms with the RT of α-pinene analytical standard (Sigma-Aldrich)



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relative percentage amounts of α-pinene recalculated according to the areas of the FID chromatographic peaks assuming that all constituents of essential oil is 100%

Results (1): amount of essential oil

- unripe cones accumulate 1.3±SD0.63% essential oil

-contents of essential oils in unripe cones varied between 0.3–4.2%

(N=110 cone-bearing *Juniperus communis)*

- leaves accumulate 0.4±SD0.14% essential oil

-contents of essential oils in leaves varied between 0.1–0.9%

(N=110 cone-bearing *Juniperus communis*)

(11)

Results (2): percentage of α-pinene

- unripe cones contained 58.0±SD14.62% of α-pinene in essential oils
- relative percentage amount of α-pinene in essential oils of unripe cones varied between 11.5–76.8%

(N=110 cone-bearing *Juniperus communis*)

- leaves contained 54.1±SD13.9% of α-pinene in essential oils
- relative percentage amount of α-pinene in essential oils of leaves varied between 10.4–75.8%

(N=110 cone-bearing *Juniperus communis*)

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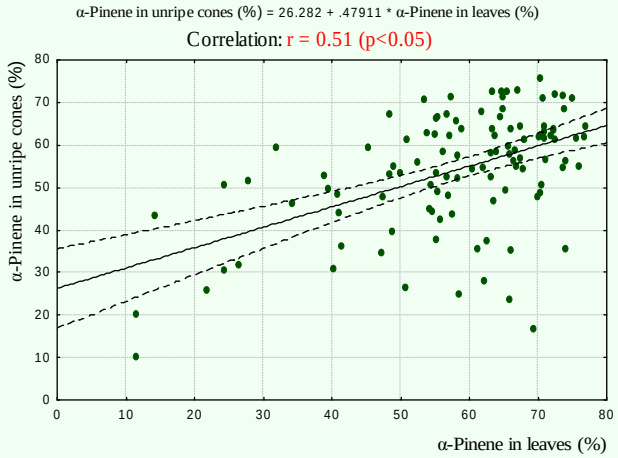
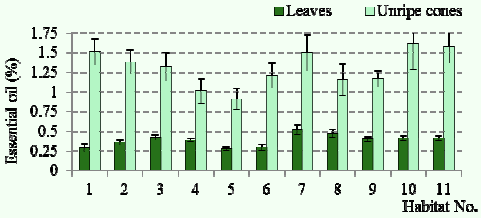
Results (3): correlations

A significant positive correlation (r=0.51, p<0.05) was observed between the percentage of α-pinene in essential oils of cones and leaves (N=110 cone-bearing *Juniperus communis*)

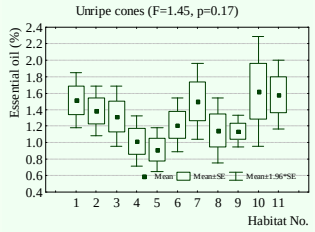


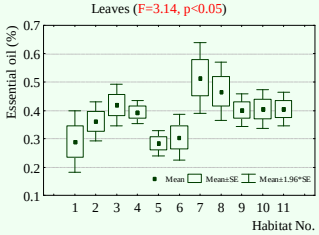
Results (4): amount of essential oil in separate habitats

The averages of amounts of essential oil in separate investigated habitats (N=11 habitats) differed nearly twice in leaves and unripe cones.

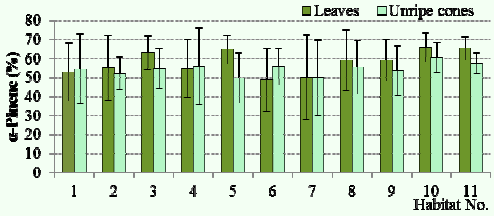


However, the test of mean comparison (ANOVA) showed the significant differences (F=3.14, p<0.05) between investigated 11 habitats by amounts of essential oils in leaves only.

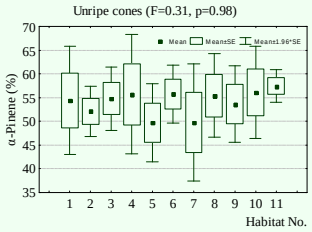


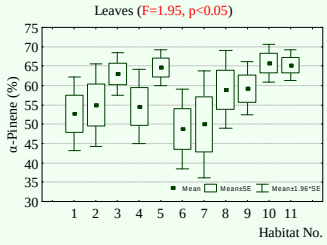


Results (5): percentage of α-pinene in separate habitats



Though the average percentages of α-pinene differed in different habitats only up to 24% and up to 18% in leaves and cones, respectively, however, the test of mean comparison (ANOVA) showed the significant differences (F=1.95, p<0.05) between investigated 11 habitats by amounts of essential oils in leaves.





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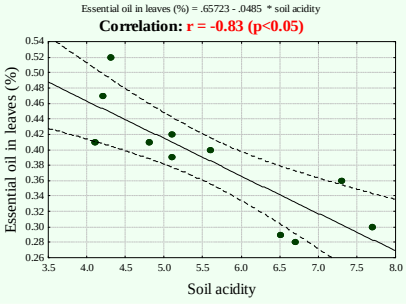
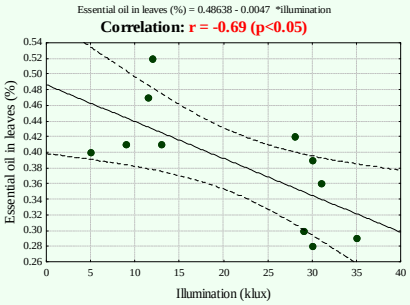
Results (6): correlations

A significant negative correlations were observed:



- between the amount of essential oil in leaves and soil acidity (N=11 habitats)



- between the amount of essential oil in leaves and illumination (N=11 habitats)

No significant correlation was detected between the amount of α-pinene and the studied ecological factors.

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Conclusions

Unripe cones accumulate more essential oils, than leaves of *Juniperus communis*

Amounts of α-pinene are similar and positively correlate between essential oils of leaves and unripe cones of *Juniperus communis*

High intraspecific variation of amount of essential oil and α-pinene is characteristic for unripe cones and leaves of *Juniperus communis*

The investigated some ecological factors influence on amount of essential oil of leaves only: the higher essential oil amounts in leaves were established in shaded habitats and habitats with acid soil

No significant correlation was detected between the amount of α-pinene and light and studied soil characteristics