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ESSENTIAL OIL OF COMMON JUNIPER (*JUNIPERUS COMMUNIS* L.) IN ALBANIA

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Ефірна олія ялівцю звичайного (Juniperus communis L.) в Албанії.– І. Саламон[1], А. Ібраліу[2], М. Кривцова[3], П. Петруска[1]. *– Рід Juniperus налічує близько 60 видів. Найбільш поширеним є ялівець звичайний (Juniperus communis L.). Основною сировиною для виробництва історично традиційного словацького алкогольного напою "Spišská borovička" є звичайні шишкоягоди ялівцю. Сьогодні шишкоягоди в основному імпортуються з Албанії, де рослини ростуть зазвичай на скельних неродючих ділянках, на полях, луках, лісосіках, у рідколіссі тощо. У 2013 році ягоди, були зібрані з 16 населених пунктів Албанії, висушені, із них вилучені ефірні олії та проаналізовані за допомогою GC/MS аналізу. У ефірній олії, вміст якої коливався від 1,2% до 3,8%, визначено від 34 до 47 речовин. Албанські рослини мають більше географічних типів, які були визначені на підставі композиції ефірної олії. Перший з них має домінуючі сполуки β-мірцен (44,5 ± 3,04%) і α-пінен (19,6 ± 3,35%). Другий тип характеризується вмістом α-пінену (25,1 ± 1,78%), β-пінену (13,4 ± 4,41%) та β-мірцену (21,2 ± 4,79%), а третій: α-пінен (31,6 ± 1,81%), β-пінен (13,6 ± 1,78%) і β-мірцен (18,5 ± 5,60%). Останній має дуже високий вміст α-пінену (37,7 ± 1,92%), β-пінену (12,4 ± 2,22%) і β-мірцену (18,6 ± 3,65%). Моніторингові дослідження різноманіття рослин ялівцю в Албанії сприяють підвищенню ефективності та покращенню виробництва спиртового дистиляту.*
Ключові слова: *ялівець, ефірна олія, GC/MS, Juniperus communis L.*
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Essential oil of common juniper (Juniperus communis L.) in Albania.– I. Salamon[1], A. Ibraliu[2], M. Kryvtsova[3], P. Petruska[1]. – *Genus Juniperus has about 60 species. The most wide-spread species is common juniper (Juniperus communis L.). The main raw material for the production of historical typical Slovak alcohol beverage ,,Spišská borovička" is common juniper fruits. Nowadays berries are mainly imported from Albania, where the plants are growing typically on the rocks, on barren grounds, in fields, grass fields, clear-cut areas, in open forests and in other places. In 2013 the fruits were collected from 16 localities in Albania, naturally dried, extracted essential oils and analysed by GC/MS. The content of essential oil varies in the range of 1.2% to 3.8% and from 34 to 47 substances was identified. The Albanian plants have more geographic types, which were identified on base of the essential oil composition. The first has the dominant compounds β-myrcene (44.5 ± 3.04%) and α-pinene (19.6 ± 3.35%). The second type is characterised by the contents: α-pinene (25.1 ± 1.78%), β-pinene (13.4 ± 4.41%) and β-myrcene (21.2 ± 4.79%) and the third: α-pinene (31.6 ± 1.81%), β-pinene (13.6 ± 1.78%) and β-myrcene (18.5 ± 5.60%). The last has very high content of α-pinene (37.7 ± 1.92%), β-pinene (12.4 ± 2.22%) and β-myrcene (18.6 ± 3.65%). This biodiversity monitoring of Albanian juniper plant population contributes for increasing efficiency and enhancement of spirit distillate production.*
Key words*: Common juniper, essential oil, GC/MS, Juniperus communis L.*
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Introduction

Common juniper, *Juniperus communis* L., is a shrub or tree species belonging to the cypress family (*Cupressaceae)*. It has wide ecological amplitude. Juniper occurs abundantly in dry sunny hillsides, as well as the subalpine level. It extended almost the entire territory in Slovakia. The human activity affected its spreading. Juniper habitats are scattered to concentrate along with other light-requiring species of plants, mostly shrubs that occur within communities of grasslands and scrubland vegetation. It expands on the extensive use of pasture land or land that is no longer used by grazing. Juniper is therefore an indicator of succession after the disappearance of grazing. The Natura 2000 project - coherent European network of protected areas, in Slovakia 66 habitats of European importance, include the

code Kr2 which note "juniper populations", quantifying of social value of 15.93 euros per 1 m^2 (Šubová et al. 2000).

Juniper berries are used also in phytotherapy, as an infusions, alcoholic extracts and essential oils (Jahodár 2010). Significant application is in the manufacture of alcoholic beverages. Essential oil is used for production typical Slovak alcoholic drink „*Spišská borovička*" (PRELIKA, Co., Presov, Slovakia). The aim of the contribution is evaluated the habitats with the juniper populations in Albania for them chemotype biodiversity.

Material and Methods

Plant material. The fruits of common juniper were collected from different individuals on 16 localities (together 64 samples) in Albania in 2013/2014.

Isolation of the essential oil. Ten grams of each sample of juniper was grounded in a blender and then subjected to hydro distillation for 2 h according to the standard procedure described in the European Pharmacopoeia (2004). The oils were solubilised in *n*-hexane and stored under N_2 at +4 °C in the dark until were analysed. The plant materials gave yellow reddish oils.

Gas chromatography and components identification. GC/MS analyses were carried out on a Varian 450-GC connected with a Varian 220-MS. The separation was achieved using a FactorFour^{TM}: Capillary Column VF 5ms (30 m × 0.25 mm i.d., 0.25 µm film thickness). Injector type 1177 was heated on temperature 220 °C. Injection mode split less (1 µL of a 1:1,000 *n*-hexane solution). Helium was used as a carrier gas at a constant column flow rate of 1.2 ml/min. Column temperature was programmed: initial temperature 50 °C for 10 minutes, then to 100 °C at 3 °C/min; isothermal for 5 minutes and then continued to 150 °C at 10 °C/min. Total time for analysis of one sample took 46.67 minutes. Identification of components were made by comparison of their mass spectra with those stored in NIST 02 (software library) or with mass spectra from the literature and a home-made library, as well as on comparison of their retention indices with the standards.

Disk diffusion method. Antimicrobial activity of essential oils was determined using disk diffusion method. Sterile filter paper disks (6 mm in diameter) impregnated with 10 µL of essential oil were placed on the dish plate previously inoculated with a microbial suspension. Bacterium inocula 100 µL in physiological solution were adjusted to the equivalent of 0.5 McFarland standard, and evenly spread on Muller-Hinton agar surface (incubated at 37±2 °C for 24 hour), yests – on SDA agar (incubated at 35±2 °C for 48 hour). The diameters of the inhibition zones were measured in millimeters including diameter of disc. Each antimicrobial assay was perfomed in at least triplicate. As test culture, the following bacteria from the ATTC (American Type Culture Collection, USA) collection were used: *Candida albicans* ATCC 885-653; *Staphylococcus aureus* ATCC 25923 F-49*; Escherichia coli* ATCC 25922 (F-50)*, Enterococcus faecalis* ATCC 29212. We used also clinical strains of bacteria and yeast *C. albicans, C. krusei, C. tropicalis, S. aureus, E. coli, Cirtobacter* spp. which were isolated from sputum of people with pneumonia, obstructive bronchitis, bronchial asthma, chronic obstructive disease and oral cavity patients with periodontal disease (Rнos et al. 2005).

Results and discussion

In 2013 the fruits were collected from 16 localities in Albania, naturally dried, extracted essential oils and analysed by GC/MS. The content of essential oil varies in the range of 1.2% to 3.8% and from 34 to 47 substances was identified. The Albanian plants have more geographic types, which were identified on base of the essential oil composition (Table 1). The first has the dominant compounds β-myrcene (44.5 ± 3.04%) and α-pinene (19.6 ± 3.35%). The second type is characterised by the contents: α-pinene (25.1 ± 1.78%), β-pinene (13.4 ± 4.41%) and β-myrcene (21.2 ± 4.79%) and the third: α-pinene (31.6 ± 1.81%), β-pinene (13.6 ± 1.78%) and β-myrcene (18.5 ± 5.60%). The last has very high content of α-pinene (37.7 ± 1.92%), β-pinene (12.4 ± 2.22%) and β-myrcene (18.6 ± 3.65%). This biodiversity monitoring of Albanian juniper plant population contributes for increasing efficiency and enhancement of spirit distillate production.

Table 1. Qualitative and quantitative characteristics of the common juniper population – essential oil in Albania

	number of samples	α-pinene	sabinene	β-pinene	β-myrcene	limonene	tepinen-4-ol	bornyl acetate	β-caryo-phylene
group No.1	8	19.57 ± 2.74	< 1	1.50 ± 0.43	44.50 ± 2.46	5.12 ± 1.02	< 1	< 1	4.25 ± 0.84
group No.2	24	25.04 ± 0.75	5.58 ± 0.89	13.42 ± 1.86	21.21 ± 2.02	4.46 ± 0.72	< 1	< 1	9.08 ± 1.38
group No.3	16	31.56 ± 0.95	4.50 ± 0.82	13.56 ± 0.95	18.50 ± 2.97	4.25 ± 0.71	< 1	< 1	5.87 ± 1.31
group No.4	16	37.69 ± 1.02	4.06 ± 0.71	12.44 ± 1.18	18.56 ± 1.93	3.87 ± 0.77	< 1	< 1	3.75 ± 1,20

The dominant compounds of juniper essential oil are: α-pinen, β-pinen and myrcen. On the basis of their content and the ratio can be divided into four samples groups:

- *The first group*: The highest content is myrcene and its average is 44,5% and the contents of α-pinene is 19.57% and β-pinene is very low (1.5%).
- *The second group:* The content of α-pinene is 25.04%, myrcene 21.21% and these results are nearly same. Quantity of β-pinene is 13.41% and in this group was detected the the highest content of caryophylene (9.08%).
- *The third and fourth group* are similar. The myrcene content is 18.5% (the 3rd group) and 18.56% (the 4th group). Quantity of β-pinene is 13.56% (the 3rd group) and 12.43% (the 4th group). The very important is the differences of the α-pinene characteristics, which are 31.56% (the 3rd group) and 37.68% (the 4th group).

This plant species extended almost the entire territory in Slovakia. In the comparison with the Albanian research, the juniper fruits were collected from three localities in Northeast Slovakia in the October 2012. The essential oil content varies in the range from 0.20 to 0.42%. The 34 to 47 chemical components were identified. Dominant compounds were α-pinene, the content ranged from 25.81% to 43.35% and β-pinene, which amount ranging from 13.29% to 20.64%.

The results of the juniper berry essential oil content and its composition from both countries present the significant variation, which is depending on the age of the plant and concrete localities.

Gonny et al. (Gonny et al. 2006) identified 22.1% amount of α-pinene and only 1.5% of β-pinene in the fruits of *Juniperus communis* L. On the other hand he determined the highest amount of limonene (49.3%). In the analyses of the Slovakian samples, the limonen amount was 0.77% in Vyrava and 5.63% in Kišovce.

Chatzopoulou et al. (Chatzopoulou et al. 1995) analysed the essential oil from the juniper grown in Greece. The essential oil was extracted after 1, 2, 3, 4, 5 and 6 hours of hydro distillation. 26.04% of α-pinene was identified after 2 hours of hydrodistillation and β-pinene was not noted. The amount of limonene was 1.37% and highest amount determined in β-caryophyllene 6.97%, sabinene 9.23% and germacrene D 12.89%. After different time period they identified different amount of some components, for example α-pinene after one hour distillation was identified 27.78% and after six hours only 9.78%.

High amount (10.9%) of germacrene D was measured by Marongiu et al. (Marongiu et al. 2006) in the juniper fruits cultivated in Sardegna, Italy. In the same sample were identified 44.0% of limonene, 2.6% of α-pinene and 0.8% of β-pinene.

Gonny et al. (Gonny et al. 2006) and Chatzopoulou et al. (Chatzopoulou et al. 1995) determined myrcene (6.30% and 9.21%). In our samples was not identified.

El-Chorab et al. (El-Chorab et al. 2008) performed two types of analyses. Its two column chromatographic fractions (eluted with hexane and ethyl ether) were analysed by gas chromatography/mass spectrometry. The major compounds in the dichloromethane extract were α-pinene 23.73%. A fraction eluted with hexane contained 44.24% α-pinene. These results could present the importance of the extraction solution.

Marongiu et al. (Marongiu et al. 2006) recorded in three different samples of juniper fruits 22.93 – 60.07% of α-pinene, 1.50 – 5.60% of β-pinene, 0.68 – 15.72% of myrcene and 0.68 – 9.96% of p-cymene.

According to results, it has been found that essential oils of *Juniperus communis* L. showed high level of antibacterial activity against *Citobacter* spp. (20.00±0.10 mm). Essential oil have also significant antimicotic activity to *Candida spp*. clinic isolates − *C. albicans* (21.00±0.20 mm), *C. krusei* (14.50±0.10 mm), *C. tropicalis* (13.00±0.15 mm). Lesser effect was registered with application of essential oil on *S. aureus*. Zones of growth retardation for the *Stathylococcus spp*. isolates varied from 7.00±0.10 mm to 7.50±0.20 mm. Essential oil revealed only bacteriostatic effect on *E. coli*: zone of growth inhibition was 16.00±0.10 mm.

Conclusion

Common Juniper, *Juniperus communis* L. is a shrub or tree species belonging to the cypress family (*Cupressaceae*) with wide ecological amplitude. The fruits were collected from 16 localities in Albania in 2013, naturally dried, extracted essential oils and analysed by GC/MS. The content of essential oil varies in the range of 1.2% to 3.8% and from 34 to 47 substances was identified. The Albanian plants have more geographic types, which were identified on base of the essential oil composition. The first has the dominant compounds β-myrcene (44.5 ± 3.04%) and α-pinene (19.6 ± 3.35%).

The obtained results suggest that the content and composition of essential oil of juniper berries (*Juniperus communis* L.) varies depending on the age of the plant and localities. For the determination of essential oil components has a significant effect distillation length as well as the method of analysis. It is necessary to assess the more detailed study of environmental factors in studied localities.

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