

Full Length Research Paper

# Composition of essential oils from three classes of juniper fruit from Serbia

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*Juniperus communis* L. (Cupressaceae) is widely distributed in the forest region of Serbia. This refers especially to degraded and devastated forests and abandoned farmlands. In addition to ecological significance as a pioneer species in forest regeneration, it is also of high economic interest. Its fruit (*Juniperi fructi*) produces a valuable essential oil (*Juniperi aetherolium*). Samples of *J. communis* fruits were collected from different places in Serbia and the content of essential oil was determined. Mature fruits were analyzed, with the content of unripe fruits up to 5%. The content of essential oils ranged between 2.30 and 2.66%.

**Key words:** *Juniperus*, fruits, essential oil, Serbia.

## INTRODUCTION

The genus *Juniperus* (Cupressaceae) which contains more than 60 species is quite widespread and grows wild all over the northern hemisphere. *Juniperus communis* L. is widely distributed throughout Europe, West and North Africa and North America. It is an amphi-boreal-mountain-continental type of distribution (Mensel, 1943), that is, circum-boreal floral geographic element. *J. communis* is a species with great ecological amplitude. In Serbia, it occurs from Hungarian oak-Turkey oak forests and forests with Eastern hornbeam at foothills, beech-fir forests and spruce forests, to devastated pastures over very diverse geological substrates and soil types. Ecological diversity of sites where juniper occurs, as well as its geological age tertiary resulted in great variability, that is, adaptation to the specific conditions (ecotypes, chemotypes, varieties, etc.). The oil of *J. communis* has been used for centauries as diuretic, based on its terpinan-4-ol content. The plant is also used in folk medicine as carnitative, antiseptic, and as remedy for indigestion. Some of antimicrobial properties of the essential oil have also been reported. *Juniperus* are widely used in compounding spices, and his oil is used in perfumes, pharmaceutical and cosmetic products. The oil

and the extracts are also used for the flavouring of beverages and liquerous (Tokin, 1951; Pashalina, 1993). Changes in the content and composition of an essential oil from Juniper can be caused by environmental factors, such as soil or climate in which the plants are grown, and by different harvesting methods or distillation techniques (Miller, 1951). As a rule, essential oil yield is lower in the regions situated north from ex Yugoslavia. The average contents of essential oil in juniper fruits were reported to be cca 2.45, 1-1.5, 1-1.2 and 0.8-1.7% in France, Germany, Chezka and Russia, respectively, and 0.2-0.5% in Serbia (Jovanović, 1992). The isolation and concentration techniques normally used may alter the quantitative as well as the qualitative composition of the obtained essential oil relative to the composition present in the plant material.

## MATERIALS AND METHODS

The samples of juniper fruit were collected at the different places in Serbia, during 2008. The essential oil was determined by steam distillation and analyzed by gas chromatography using a flame ionization (FID) detector (Adams, 1995). The Hewlett-Packard gas chromatograph was used, model HP 5890 Series II, fitted with split-splitless injector. Two sistem of columns were connected with chromatographic integrator: *Column Ultra 1* (50 m × 0.32 mm, film thickness 0.52 μm). The sample was dissolved in hexane and injected in the split mode. The injector temperature was 250°C, and

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**Table 1.** Content of essential oil in juniper fruits after distillation.

Juniper fruit quality	Moisture %	Essential oil (%)	
		Fresh	dry
I class	20.29	2.12	2.66
II class	27.79	1.68	2.31
III class	22.50	1.78	2.30

**Table 2.** Physico-chemical characteristics of essential oils from Juniper fruit.

Character	<i>Juniperi fructus</i> I class	<i>Juniperi fructus</i> II class	<i>Juniperi fructus</i> III class
Colour	Colourless	Colourless	Colourless
Transparency	Clear	Clear	Clear
Relative density	0.8541	0.8638	0.8744
Refraction index	1.4739	1.4766	1.4806
Optical rotation	-0.35	-1.8	-4.7
Ester value	1.00	6.89	9.26
Acid value	0.57	0.91	0.70

detector temperature was 300°C in the linear temperature program 40 to 280°C/min. The quantification was based on area of percentage report obtained by GC-FID. Qualitative analysis of essential oil constituents was performed by a mass-spectrometry detector using Chromatograph HP 5890 Series II, HP 5971 MSD and electron impact mode (70 eV). *Column Pona* (50 m × 0.2 mm, film thickness 0.5 µm). The sample was dissolved in hexane and injected in the same temperature program. The transfer line temperature was 280°C. The components were identified by comparing the retention times and mass spectra of the components with the standards as well as by comparing mass-spectra with Wiley/NBS data base of mass spectra. Physical and chemical characters of the oil samples were studied according to ISO for juniper (*J. communis* L.) essential oil (Tucakov, 1964). Juniper fruits were classified into three quality classes according to percentage of moisture and the content of essential oil was determined by distillation for 2 h after Clavenger (Regul. Ph. Jug. IV).

## RESULTS AND DISCUSSION

Content of essential oil in juniper fruits are presented in Table 1. Obtained results show that the content of essential oils was the highest in the Class I (2.66%) that had the lowest percentage of moisture. These results agree with the values obtained by long-term examination in Serbia (Matović, 1996; Matović et al., 1997, 1998). Therefore, regarding the content of essential oil all the three classes can be considered for good quality for the intended purpose. The results of the analysis of physico-chemical characteristics of essential oil obtained from the three classes of juniper fruits are presented in Table 2. Toward the colour, transparency, relative density and refraction index these classes were very similar. But, differences existed in optical rotation, ester value and acid value. Along with the quantitative analysis of the first, second and third classes of juniper fruits used in

essential oil manufacture in Serbia, we performed the qualitative analysis as well as the identification of significant essential oil constituents (in the three samples) and the quantitative determination of the prevailing constituents. Many researchers have examined the composition of essential oil of Juniper in different places of the world (Ramic and Murko, 1983; Angio ni et al., 2003; Sawai et al., 2007). In the essential oil from *J. communis* from Serbia was registered and quantified 15 components (Table 3). It was found that  $\alpha$ -pinene was the major constituent of essential oils (the greatest amounts at all of classes). Sabinene and  $\beta$ -myrcene were also found in considerable amounts. Except those, in larger quantities was identified terpinene-4-ol.

This composition of essential oil of juniper is, largely, the result of specific environmental factors in the area from which fruits were collected. Namely, Juniper fruits originated from southwestern Serbia (particularly from Zlatar mountain and its slopes). The average altitude of this mountain is 1230 m. The climate is submountain with fresh summers and mild winters. Autumn is warmer than spring. Zlatar is characterized by the highest number of sunny days in Serbia (285) and mean annual temperature is 17°C. The air is rarefied and air pressure is low. Mixture of influences of Mediterranean and mountain climate acts as a natural healing factor. On the mountain represented dense evergreen and birch forests. Numerous forest species were found of Zlatar Mt (*Abies alba* Mill., *Pinus silvestris* L., *Acer platanoides* L., *Betula verrucosa* Ehrh., *Carpinus betulus* L., *Crataegus monogyna* Jacq., *Cornus mas* L., *Juglans regia* L., *J. communis* L., *Mentha longifolia* (L.) Huds., *Ribes nigrum* L., *Rhus cotinus* L., *Quercus pubescens* Willd) (Matović, 1996). The study reveals that they are the aromatic

**Table 3.** Composition of essential oils from three classes of Juniper fruit from Serbia.

No	Component	Class	Rt <sub>min</sub>	Percentage
1	a-Thujene	Class I	16.1	1.39
		Class II	16.3	1.17
		Class III	16.8	1.27
2	a-Pinene	Class I	16.5	16.65
		Class II	16.8	23.56
		Class III	17.3	27.82
3	Camphene	Class I	17.4	0.22
		Class II	17.5	0.21
		Class III	18.1	0.23
4	Sabinene	Class I	19.0	14.54
		Class II	19.1	10.8
		Class III	19.7	12.90
5	b-Pinene	Class I	19.2	1.94
		Class II	19.4	1.65
		Class III	20.0	1.88
6	b-Myrcene	Class I	20.3	14.17
		Class II	20.5	13.45
		Class III	21.1	11.18
7	Limonene	Class I	23.0	3.50
		Class II	23.2	4.51
		Class III	23.8	3.89
8	g-Terpinene	Class I	25.1	0.89
		Class II	25.3	0.98
		Class III	25.9	1.03
9	a-Terpinolene	Class I	27.3	0.91
		Class II	27.6	0.86
		Class III	28.1	0.98
10	Terpinen-4-ol	Class I	33.4	3.74
		Class II	33.6	8.17
		Class III	34.1	4.65
11	a-Terpineol	Class I	34.2	0.29
		Class II	34.4	0.80
		Class III	35.0	0.58
12	a-Humulene	Class I	51.2	2.36
		Class II	51.4	2.66
		Class III	51.9	1.69
13	Germanene D	Class I	53.3	2.38
		Class II	53.5	2.67
		Class III	54.0	1.71
14	g-Cadinene	Class I	54.7	0.27
		Class II	54.9	0.33
		Class III	55.4	0.22
15	d-Cadinene	Class I	55.0	8.65
		Class II	55.2	8.00
		Class III	55.7	6.42

species of trees, shrubs and herbaceous plants have an antimicrobial effect. These results are presented in Table 3 and Data from Tables 1 and 2 compared with the standards for the essential oil of juniper, gave the following results: a) the essential oil obtained by distillation of the first class of drugs *Juniperi fructus* not responsible for its physical and chemical characteristics of standards for essential oil of juniper. The reason is the positive (+) angle turns. (b) The essential oil obtained by distillation of a sample of second class of drugs *J. fructus* is not meet standard for its physical and chemical characteristics. The reason is the positive (+) angle turns, too (c) The essential oil obtained by distillation of samples of the third class of drugs *J. fructus* fully meets the standards for its physical and chemical characteristics. As a result of this study, the essential oil obtained by distillation of the different samples of *J. fructus* (III class) available in Serbia corresponds to all physical and chemical standards for juniper essential oil (Matović, 1997). *J. communis*, therefore, was considered as significant economic potential in Serbia.

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