

REVIEW PAPER

Characterization of the essential oil from cone-berries of *Juniperus communis* L. (Cupressaceae)

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Summary

Juniperus communis L. (Cupressaceae) is a plant widely cultivated in the Northern hemisphere. Juniper berries, the fruit of *Juniperus communis* L. are a highly valued, essential oil-rich plant material used traditionally in folk medicine as antiseptic, diuretic, antirheumatic, anti-inflammatory, antibacterial and antifungicidal agent. This paper reviews information on extraction methods of the essential oil from the juniper berries, its chemical composition and antimicrobial as well as antioxidant properties.

Key words: *Juniperus communis* L., essential oils, *Juniperi pseudofructus*

INTRODUCTION

Juniperus L. (consisting of approximately 70 species and 40 varieties) is the second most diverse genus of the conifers. The genus is divided into three sections, and one of them is *Juniperus* (syn: sect. *Oxycedrus* Spach), containing 12 species. *Juniperus communis* L. (Cupressaceae Rich. ex Bartl.), a highly variable taxon distributed in Northern hemisphere (including Baltic Sea region), has the largest distribution of all juniper species.

The cone-berries of *J. communis* (known as *Juniperi pseudofructus*) are used since ancient

times in folk medicine to cure cystitis, digestive disorders, in therapy of chronic arthritis and other indications. The berries contain essential oil with characteristic conifer-like aroma and bitter taste. Many constituents present in juniper essential oil are responsible for the oil biological properties. Antibacterial and antifungal properties of the essential oils as well as of oil constituents are well documented [1]. Essential oil or some of its constituents have found application as antimicrobial agents for food preservatives, in clinical microbiology or in pharmaceutical preparations. The juniper essential oil is used in many

industries, for example in food industry to flavor alcohols such as gin or in production of blended teas. The health benefits of juniper essential oil can be attributed to its properties as an antiseptic, sudorific, antirheumatic, depurative, antispasmodic, stimulating, stomachic, astringent, carminative, diuretic, rubefacient, vulnerary and tonic substance.

Commercial juniper berry essential oil is rarely a true distillate from berries and may be a by-product from gin or brandy manufacture. In addition to berries, also branches, needles and wood of juniper contain essential oils. Juniper needles contain 0.2–1.0% of volatile oil. Oil yield depends on the degree of ripeness, seasonal variations, environmental conditions (temperature, sunlight, photoperiod), age of plant latitude and altitude of growing site, a role in selective browsing damage by local herbivores and other factors. The average yield of the essential oil varies from 0.47 to 0.75% in dried needle with young juniper branches and 0.1–0.28% in dried branches according to the month of collection [2, 3]. The most significant changes in the content of the oil were found in spring–summer period of vegetation.

There is no monograph concerning juniper needles or branches in European Pharmacopoeia (EP) [4], but the monograph of *Juniperi pseudofructus* describes them as the ripe cone-berry of *J. communis* L., which may not contain less than 10 ml/kg of essential oil. The amount of the essential oil can be up to 3%.

Botanical aspects

J. communis is an evergreen, perennial, long-lived (to 600 years or more) coniferous plant having the largest range of any woody plant in the cool temperate geographical regions of Northern Hemisphere, from the southern part of the Arctic, in mountains, to around a latitude of 30° north in Europe, Asia and North America. *J. communis* is a globally distributed species exhibiting a wide range of ecological adaptations. A wide geographical distribution is the main reason for the remarkable variation in the morphological characteristics and chemical composition of the secondary metabolites of *J. communis* [5].

J. communis has green and sharp leaves (needles) in whorls of a three; the leaves remain on the branch for up to 4 years. *J. communis* is a dioecious species: male and female cones grow

on separate wind-pollinated plants. The plant blooms in April–May, however the cones of *J. communis* maturation time is late autumn of the second year. Therefore, the unripe second year and ripe third year berries may be collected from the same plant simultaneously. The spherical cones are berry-like, blue-black with a waxy coating and usually have three (or sometimes six) scales, each scale with a single seed. *J. communis* is a very slowly growing plant reaching approximately 20 and 50 cm in height after 5 and 10 years, respectively [5].

The cultivation of *J. communis* is not very problematic. The maintenance of plant is low, it prefers full sun and a well drained, slightly acidic soil. This popular garden shrub is resistant to low temperature, harsh weather conditions and environmental pollution.

Extraction of essential oil from berries

Most of the organs of *J. communis* contain essential oil, but is extracted mainly from berries, needles and branches. Traditionally, the oil is collected by extraction using organic solvent: methanol, n-hexane, but the main process applied is distillation of the crushed, dried, partially dried or fermented berries [6].

Juniper essential oil is usually present in berries at relatively low concentrations (0.2–3.42%) and recovery techniques of high performance are required to achieve high oil yields. Various techniques have been used for juniper oil extraction such as hydrodistillation [7, 8], supercritical carbon dioxide extraction [9–12], solvent extraction [10] and simultaneous distillation extraction method [8].

Damjanović *et al.* isolated volatile compounds from berries of common juniper by three different techniques: hydrodistillation, hexane extraction and supercritical CO₂ extraction [10]. They obtained the essential oil with yield of 2.17% using hydrodistillation, the hexane extraction yield was 5.31% and supercritical CO₂ extraction yield was 0.96%. Chemical composition analyses conducted by GC/MS revealed that the samples differed quantitatively and qualitatively. The concentrations of monoterpene hydrocarbons (α -pinene, sabinene, myrcene) were higher in the hydrodistilled oil, while some less volatile compounds were present in extracts, especially in hexane extract.

Each technique of extraction has particular advantages and disadvantages. Nevertheless, juniper oil is most frequently obtained by hydrodistillation. A Clevenger-type hydrodistillation apparatus is normally used for the recovery of juniper oil in the laboratory, while different types of water, water-steam and direct steam distillation units of different size and design are applied for industrial recovery. Hydrodistillation not only produces high quality juniper oil, but it is also relatively simple and safe to operate, as compared to other extraction techniques. It is also environmental friendly [6].

In the last decade, classic hydrodistillation has been improved by involving microwave irradiation to heat the water suspension of the plant material. Microwave-assisted hydrodistillation (MAHD) has been widely used to isolate essential oils from various plant materials [13-15]. In MAHD the sample reaches its boiling point very rapidly, leading to a very short extraction. In addition, with the microwave distillation technique it is possible to achieve distillation with the indigenous water of the fresh plant material. The use of MAHD at present is limited to laboratory scale, and its future industrial application is dependent on the scalable design of functional microwave equipment, which requires greater investment and better process quality control.

Chemical composition of juniper berry essential oil

The chemical composition of essential oils obtained by hydrodistillation from juniper leaf (needles) [16-20] and berries [19-22] has been studied by several authors.

Dried berries of *J. communis* contain 30–40% of sugars (mainly glucose and fructose), 1.2–10% resin and considerable amounts of organic acids and essential oils (0.2–3%).

According to monographs of juniper oil (*Juniperi aetheroleum*) in European Pharmacopoeia [4], the essential oil obtained by steam distillation from the ripe, non-fermented berry cones of *J. communis* L. and the percentages of the components are placed within following ranges: α -pinene 20–50%, sabinene less than 20%, β -pinene 1–12%, β -myrcene 1–35%, α -phellandrene less than 1%, limonene 2–12%, terpinen-4-ol 0.5–10%, bornyl acetate less than 2%, and β -caryophyllene less than 7%.

Using GC/FID and GC/MS assays, 70 compounds were identified in the essential *J. communis* oil, although, some reports claim that there are more than 100 chemical compounds [23]. The composition of the essential oil varies upon origin of berries but consists mainly of monoterpene hydrocarbons (about 60% of the essential oil). The major constituent of the unripe and ripe berry essential oils of *J. communis* from European countries is α -pinene [10, 16, 17, 22, 24-26], (tab. 1). The essential oils may comprise about 35% to 57% of α -pinene. The second most abundant constituent of the essential oil is sabinene which content ranges from 0.17% in oil from Olimp (Grece) to 28.8% in oil from sabinene chemotype berries [26]. Juniper berry oils of sabinene chemotype have been found only in high mountains. The juniper essential oil is also rich in myrcene which may usually constitute 8–20% of volatiles. Myrcene is the main chemical compound which allows the differentiation between berry oil and needle oil: the needle oil may comprise only up to 5% of myrcene, whereas berry oil contains it much more, up to 20% [27]. The major oxygenated terpenoids found in juniper oil is terpinen-4-ol (0.05–2.60%). Berry essential oil contains also sesquiterpenes which amount from about 2% in oil from Macedonia (Greece) to about 10% in oil from Olimp (Greece).

Most of the monoterpene hydrocarbons found in the juniper berry essential oil show optical activity, but only few articles describe enantiomeric composition of the oil. Sabinene and β -pinene exist only in one enantiomeric form, sabinene as the dextrorotary (+)-form and β -pinene as the laevorotary (-)-form [28, 29]. α -pinene appears in both enantiomers. In the oil from berries collected in northern Poland (R)-(+)- α -pinene prevails at comparable levels, varying from 33 to 62%, whereas in the oil from France and different locations in Poland (-)- α -pinene dominates. In the berry essential oil a large excess of (+)-limonene over its (-)-antipode is observed, which seems to be characteristic of the *Rutaceae*, while for essential oils from the *Pinaceae* (-)-limonene is most abundant [28].

For comparison, table 1 summarizes chemical composition of juniper berries oil from several European countries. Changes in the composition of essential oil can be caused by environmental factors, such as soil or climate conditions as well as by different harvesting methods or distillation techniques.

Table 1.
Percentage composition of selected compounds found in the essential oil of cone-berries of *Juniperus communis*

Compound	Geographical origin of berries						
	Lithuania [24]	Sardinia [16]	Montenegro [10]	Greece Olimp [25]	Greece Macedonia [26]	Commercial product from Bulgaria [17]	Commercial product from Germany [22]
Monoterpene hydrocarbons [%]							
α -Thujene	0.00	0.53	0.10	0.08	-*	0.90	0.70
α -Pinene	43.50	52.26	39.20	42.55	57.06	51.4	35.4
Camphene	0.20	0.22	0.20	0.21	0.31	0.80	0.50
Sabinene	1.10	5.58	17.8	0.17	6.08	5.80	7.60
β -Pinene	1.60	2.86	0.00	1.65	3.41	5.00	3.30
Myrcene	19.60	15.32	18.20	8.10	10.74	8.30	15.3
α -Phellandrene	0.00	0.00	0.10	traces	0.40	-	-
β -Phellandrene	0.40	0.00	-	0.11	-	0.50	-
δ -3-Carene	0.00	0.00	1.00	0.02	0.02	0.20	0.10
α -Terpinene	0.00	0.00	-	0.02	0.12	0.10	0.50
<i>p</i> -Cymene	0.00	0.25	0.70	0.11	0.08	0.90	2.10
Mimonene	2.40	3.11	5.20	0.83	1.73	5.10	7.30
γ -Terpinene	0.40	0.52	1.40	0.05	0.34	0.20	1.80
Terpinolene	0.10	0.49	-	0.41	0.62	0.40	1.20
Total	69.30	81.14	~83.9	54.31	~80.91	~79.6	~75.8
Derivatives of monoterpenes [%]							
Linalool	0.00	0.00	0.10	0.11	0.26	0.10	0.20
Terpinen-4-Ol	0.40	1.51	2.60	0.74	0.05	0.90	2.40
α -Terpineol	0.50	0.00	0.20	0.57	0.85	0.20	0.20
Citronellol	0.00	0.00	-	0.20	5.06	-	-
Camphor	0.00	0.00	-	0.53	-	-	-
Bornyl acetate	0.80	0.66	0.20	0.59	1.08	0.30	0.20
Total	1.70	2.17	~3.10	2.74	~7.30	~1.50	~3.00
Sesquiterpenes [%]							
α -Copaene	0.00	-	-	-	-	0.50	0.50
α -Cubebene	0.00	1.25	0.40	1.29	-	0.60	0.50
β -Caryophyllene	0.10	0.00	1.00	0.77	1.86	2.00	4.20
α -Humulene	0.30	0.81	0.70	0.72	-	1.30	1.20
Germacrene-D	0.40	6.69	3.80	3.99	-	1.10	1.80
α -Muurolole	0.30	0.00	0.20	0.52	-	0.40	-
γ -Cadinene	0.60	-	0.20	0.80	-	0.50	-
δ -Cadinene	2.10	-	0.70	2.61	-	0.80	1.50
Total	3.80	~8.75	7.00	10.70	~1.86	7.20	~9.70

*the dash means no information given by the authors

Antimicrobial activity of juniper berry essential oil

The essential oil from cone berries of *J. communis* showed antimicrobial activity of Gram-positive bacteria: *Bacillus cereus* ATCC 11778, *Bacillus subtilis*

NCTC 8236, *Micrococcus flavus* MFBF, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* MFBF, *Staphylococcus epidermidis* MFBF, *Enterococcus faecalis* MFBF. The antimicrobial activity was determined by the diffusion method and the inhibition zones of tested

bacteria ranged from 10 to 16 mm [30]. Gram-negative bacteria were also sensitive to juniper essential oil. The bacterial strains such as *Serratia* spp. MFBB, *Salmonella enteritidis* MFBB, *Proteus mirabilis* MFBB, *Shigella sonnei* MFBB, *Klebsiella oxytoca* MFBB and *Yersinia enterocolitica* MFBB had inhibition zones from 8 to 17 mm. *Citrobacter freundii* MFBB and *Escherichia coli* MFBB remained resistant to the essential oil. MIC for the bacterial strains were very high, ranging from 8 to 70% (v/v) of essential oil.

Pepeljnjak *et al.* also tested antifungal properties of the essential oil from cone berries of *J. communis* [30]. *Candida albicans* MFBB, *C. krusei* MFBB, *C. tropicalis* MFBB, *C. parapsilosis* MFBB, *C. glabrata* MFBB, *C. kefyr* MFBB, *C. lusitaniae* MFBB, *Cryptococcus neoformans* MFBB, *Geotrichum candidum* MFBB and *Hansenula anomala* MFBB were sensitive to the essential oil, and the inhibition zones varied from 8 to 17 mm. Among the dermatophyte species tested, *Microsporum gypseum* MFBB and *Trichophyton rubrum* MFBB showed inhibition zones between 10 and 14 mm. Yeast, yeast-like fungi and dermatophytes were found to be very sensitive to juniper essential oil, with MIC values lower than 10% (v/v). The lowest values of MIC of the essential oil against fungal strains indicate that the main compounds present in the oil-terpene hydrocarbons (pinene, sabinene, mircene and limonene) had stronger antifungal activity than the antibacterial.

Essential oil of *J. communis* growing wild in Kosovo showed antimicrobial activity against *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *Hafnia alvei* PTCC 2005 but was not active against *Pseudomonas aeruginosa* ATCC 27853 [31].

The mechanism of antimicrobial action of terpenes is not fully understood but it is speculated to involve membrane disruption by the lipophilic compounds [32]. Antimicrobial activity of different fractions of the essential oil of *J. communis* showed that fractions containing high concentrations of α -pinene and sabinene effectively inhibited the growth of microorganisms. The fractions containing pure α -pinene and a mixture of α -pinene and sabinene successfully inhibited all bacterial strains especially the growth of fungi and yeast.

According to the experiments conducted by Filipowicz *et al.* it can be assumed that the antimicrobial effect of the juniper berry oil is the result of synergistic co-operation of a few constituents present in the oil in exceptional concentrations, namely

(-)- α -pinene, *p*-cymene and β -pinene [29]. Further studies are required to explain this phenomenon entirely.

Antioxidant activity of juniper berry essential oil

The antioxidant activity of essential oils from different juniper berry species has been established *in vitro* [33]. Anti-radical activity depends on the oil components, namely their chemical nature and concentration [34]. Regardless of its origin, juniper berry essential oil contains predominantly terpene hydrocarbons. In many cases, the essential oil antioxidant activity cannot be attributed to the dominant compounds α - and β -pinene. These monoterpene hydrocarbons in juniper berry essential oil do not contribute to a significant inhibition of malondialdehyde formation [35]. The carriers of antioxidant properties in relation to lipid peroxidation in both its stages are α - and γ -terpenes and, to a significantly lesser extent, their sesquiterpene analogues. The activity of α - and γ -terpenes is comparable to that of α -tocopherol. This has been established both for juniper essential oils [34] and for pure terpene hydrocarbons: terpinolene, α -terpinene and γ -terpinene [35]. Myrcene, α - and β -pinene only exhibit lipid peroxidation in the second stage; sabinene, limonene, α -pinene and myrcene demonstrate anti-radical activity in relation to DPPH radical. Mainly β -pinene and limonene are responsible for the scavenging effect of OH \cdot and the protection of deoxyribose against degradation. The O $_2^{\cdot -}$ neutralization is determined by germacrene-D [33].

The antioxidant capacity of the essential oil from cone-berries of *J. communis* was evaluated *in vitro* by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation scavenging, hydroxyl radical (OH \cdot) scavenging and chelating capacity, superoxide radical (O $_2^{\cdot -}$) scavenging and xanthine oxidase inhibitory effects, hydrogen peroxidase scavenging [17, 22]. The antioxidant activity of the oil attributable to electron transfer made juniper berry essential oil a strong antioxidant, whereas the antioxidant activity attributable to hydrogen atom transfer was lower. Lipid peroxidation inhibition by the essential oil in both stages, *i.e.*, hydroperoxide formation and malondialdehyde formation, was less efficient than the inhibition by butylated hydroxytoluene (BHT). These effects were confirmed in *in vivo* studies in which model organism *Saccharomyces cerevisiae* was used [17, 22]. The compounds present in juniper

berry essential oil blocked the oxidation processes in yeast cells by increasing activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GP_x). The biological effects of juniper berry essential oil *in vivo* were directly dependent on the concentrations applied.

Ethical approval: The conducted research is not related to either human or animal use.

Conflict of interest: Authors declare no conflict of interest.

CONCLUSIONS

The juniper berry essential oil has a rich history of traditional uses and benefits. The essential oil is usually obtained by hydrodistillation. Chemical composition of the oil has a huge range of variability (both quantitative and qualitative) depending on plant origin and individual plant level as well. The oil is mainly composed of monoterpenes, including α -pinene, myrcene and sabinene as major components, lesser amounts of sesquiterpenes and other volatile compounds are found. The berry essential oil shows antimicrobial and antioxidant activity, which is applied in food processing, the pharmaceutical and cosmetic industries.

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Charakterystyka olejku eterycznego uzyskiwanego z szyszkogąd jałowca pospolitego *Juniperus communis* L. (*Cupressaceae*)

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Streszczenie

Jałowiec pospolity *Juniperus communis* L. (*Cupressaceae*) jest rośliną powszechnie występującą na całej półkuli północnej. Szyszkogoda jałowca jest surowcem roślinnym dostarczającym cennego olejku eterycznego wykorzystywanego głównie w lecznictwie jako środek o właściwościach bakteriobójczych, moczopędnych, przeciwzapalnych, przeciwreumatycznych oraz przeciwgrzybiczych. W pracy przedstawiono charakterystykę olejku eterycznego uzyskiwanego z szyszkogąd jałowca pospolitego, metody jego ekstrakcji, skład chemiczny oraz właściwości przeciwbakteryjne i przeciwgrzybicze, jak również przeciwutleniające.

Słowa kluczowe: *Juniperus communis* L., olejki eteryczne, szyszkogoda jałowca pospolitego