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Research Paper

# Antimicrobial and antioxidant activity of Juniper galbuli essential oil constituents eluted at different times

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ABSTRACT

Junipers (*Juniperus* spp.) are some of the most widespread species in the world with a wide ecological adaptation. Juniper galbuli (cones or berries) and their extracts have extensive applications in pharmaceuticals, perfumery, aromatherapy, alcoholic beverages, and cooking. The objective of this study was to evaluate EO composition, antimicrobial and antioxidant activity of EO fractions captured at different timeframes during the hydrodistillation of *Juniperus communis* and *J. excelsa* galbuli. The EO fractions were captured at eight sequential timeframes after the beginning of the hydrodistillation: (0–3, 3–5, 5–10, 10–20, 20–40, 40–80, 80–160, and 160–240 min). The results showed that essential oil fractions from 80 to 160 and 160 to 240 min had 2–5 times greater antioxidant capacity than fractions captured at the beginning of the distillation or from the whole oil. The strongest antimicrobial activity of *J. communis* EO against *Salmonella enterica* subsp. *enterica* was observed in the EO obtained at the 0–3 min distillation timeframe (DT, in minutes). The EO of *J. communis* obtained at the 0 to 3, 3 to 5, and 80 to 160 min DTs showed greater antimicrobial activity against *Klebsiella pneumonia*, compared with the EO obtained from the 160 to 240 DT. The strongest activity of *J. communis* EO against *Staphylococcus aureus* subsp. *aureus* and *Candida glabrata* was observed with EO from the 160 to 240 DT. *J. excelsa EO* from the 0 to 3 and 5 to 10 min DTs had greater activity against *S. enterica* and *K. pneumonia* compared with the EO from the 160 to 240 DT. Conversely, the *J. excelsa* EO from the 160 to 240 min DT had greater activity against *Clostridium perfringens* and *Candida glabrata*. This research revealed that EO with different profiles could be obtained from the same batch of galbuli, suggesting the possibility of generating natural unadulterated oils with specific targeted profiles. Essential oils with a high content of α-pinene, β-pinene, β-myrcene, sabinene, or limonene was obtained from *J. communis* galbuli hydrodistilled for 3 min. Essential oil with a high content of α-pinene, β-pinene, β-myrcene, or γ-terpinene was obtained from *J. excelsa* galbuli when hydrodistilled for 3 min. The galbuli of *J. communis* and *J. excelsa* can yield essential oil fractions with novel chemical composition and bioactivity.

## 1. Introduction

Junipers are some of the most widespread and adapted species in the world, and are widely distributed across much of the northern hemisphere (Adams, 2011). In the European flora, there are 10 juniper species (Franco, 1964), 6 of which are also found in the Bulgarian flora; *Juniperus communis* L. (common juniper), *J. oxycedrus* L. (cade or red juniper), *J. sibirica* Burgsd. (Siberian juniper), *J. sabina* L. (savin or Cossack juniper), *J. pygmaea* C., (Alpine juniper, syn. *J. communis var. saxatilis* Pall), and *J. excelsa* M. Bieb. (forest or Grecian juniper) (Yordanov et al., 1963). Of these, *J. communis*, *J. oxycedrus*, and *J. sibirica* are widely dispersed, whereas *J. sabina* and *J. excelsa* have a limited distribution range (Gonny et al., 2006; Assyov et al., 2012; Cabral et al., 2012). *J. sabina* and the habitats of *J. excelsa* are included

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in the Bulgarian Red Book categories as endangered and critically endangered, respectively (Stoeva, 2015). These two species are included in Appendix № 2 of the biodiversity of Bulgaria (Durjaven Vestnik, State Gazette, №77, 2002).

Juniper biomass and juniper galbuli (berries, fleshy cones formed by coalesced scales into a single unit with a unified epidermis) and their extracts have wide applications in folk medicine, pharmaceuticals, perfumery, aromatherapy, food preparations and alcoholic beverages (Barjaktarovic et al., 2005; Madej et al., 2014). Juniper essential oil (EO) contains many different chemical constituents from the group of pure and oxygenated monoterpenes and sesquiterpene hydrocarbons (Pavicevic et al., 2016). The terpene compounds present in the oil are responsible for the anti-inflammatory, antimicrobial, antifungal, and antiseptic activities of the juniper galbuli EO (Filipowicz et al., 2003; Pavićević et al., 2017). Chemical composition of the juniper galbuli may also play a role in the species distribution. Junipers 'invade' pastures quickly, especially in dryer regions, to establish themselves at various altitudes ranging from 0 to 3000 masl. Although junipers are a source of natural products, contain EO, and are important species for wildlife natural habitats, some countries and individual states (such as Oregon) adopted programs for juniper eradication.

There is some information on the chemical profile of juniper galbuli EO, mainly for *J. communis* (Barjaktarovic et al., 2005). However, data on EO chemical composition from published research are difficult to compare because in most instances, authors use various extraction methods to obtain the EO from the juniper galbuli (Avci and Bilir, 2014; Pavićević et al., 2017; Sela et al., 2015). How EO compounds of juniper galbuli oil are eluted at different time intervals during the traditional distillation process is not known. It has been reported that the chemical profile of juniper leaf oil within a single species may depend on a number of factors, including the presence of chemotypes (e.g., Cantrell et al., 2013 reported 10 chemotypes within a single species of *J. virginiana*), geographical region (Gawde et al., 2009; Sela et al., 2015; Zheljazkov et al., 2017), timing of sampling throughout the year (Shanjani et al., 2010; Zheljazkov et al., 2012, 2013b; Avci and Bilir, 2014), sex of the tree (Zheljazkov et al., 2013a), distillation time (Zheljazkov et al., 2012; Cantrell et al., 2014), distillation technique (Chatzopoulou et al., 2002), genetics, and variations within the environment. However, there is no information on how the above factors would alter the EO profile of juniper galbuli oil and how the oil fractions would differ. The hypothesis of this study was that fractions of juniper galbuli oil captured at different timeframes during the extraction process would have significantly different composition and bioactivity. If the hypothesis is confirmed, then the best distillation time for each EO component will be determined so that the distillation time of the fractions can be customized for specific applications and new product development in industries utilizing juniper oil. Therefore, the objective of this study was to evaluate EO composition and antimicrobial and antioxidant activity of fractions captured at different timeframes during the hydrodistillation extraction of the galbuli of two species: *J. communis* and *J. excelsa*.

## 2. Materials and methods

### 2.1. Plant material

*Juniperus communis* galbuli were collected above the village of Markovo, near Plovdiv, Bulgaria, N4202′32.8″ E2442′07.0″ at 600 masl whereas the *J. excelsa* galbuli were collected in a National Protected Area of Tisata near the town of Kresna, Bulgaria (N41046′02.1″ E23008′59.5″ at 285 masl) in 2016. Tisata Protected Area was established in 1949 specifically to protect *J. excelsa*, and currently includes the largest *J. excelsa* concentration in Bulgaria in approximately 574 ha. The Tisata Protected Area consists of two sections, east and west, and is in the Pirin mountain foothills, 250–700 masl, and Maleshevska Planina, north of the town of Kresna. Because public access and grazing are prohibited in the Tisata Protected Area, we obtained a sampling permit from the Bulgarian Ministry of the Environment (Permit #690 of 2016 issued to Dr. Ivanka Semerdjieva and Dr. Valtcho D. Zheljazkov) to collect samples from the protected area. The galbuli collection in Tisata Protected Area was facilitated by the park rangers from National Park Central Balkan, who drove us to the area and helped with the galbuli collection. For both species, galbuli were collected from at least 10 different juniper plants to generate a representative sample of galbuli within a species and to negate possible differences in chemical compositions between individual juniper plants. The collected galbuli of the two juniper species were spread out in a layer approximately 2 cm deep in a well-aerated shady place to dry for a month at room temperature before the oil was extracted. Voucher specimens of these species were deposited at the Herbarium of the Agricultural University, Plovdiv, Bulgaria (SOA) (Thiers, 2012).

### 2.2. Essential oil (EO) extraction of the juniper galbuli

The EO of the galbuli was extracted via hydrodistillation in 2-L distillation units (Laborbio Ltd Sofia, laborbio.com), and each extraction was performed in three replicates. Prior to the extraction, 100 g of galbuli plus 1.2 L of water were placed in a kitchen food processor (blender) and ground for 48 s. Grinding juniper galbuli in water has two purposes: (1) grinding greatly facilitates EO extraction, reducing substantially the time and energy needed for extraction; and (2) grinding in water eliminates EO losses due to volatilization during the grinding process.

The beginning of the distillation in each replicate was marked when the first droplet of EO dropped from the condenser into the collecting unit (Florentina) of the apparatus. The EO fractions were captured at eight timeframes from the beginning of the distillation: (0–3, 3–5, 5–10, 10–20, 20–40, 40–80, 80–160, and 160–240 min). The oil fractions were captured without interrupting the hydrodistillation process, resulting in eight successive fractions representing the eluted oil constituents within these timeframes. The oil was measured by volume, transferred in 2-mL vials and placed in a freezer until the oil was analyzed.

### 2.3. Gas chromatography (GC)–mass spectroscopy (MS) analyses of the essential oil (EO) fractions

The EO fractions from the galbuli of the two juniper species, captured in eight different timeframes and three replications were analyzed for chemical profile by GC-FID and GC/MS techniques (Adams, 2007). The GC/MS analysis was carried out with an Agilent 5975C MSD system coupled to an Agilent 7890A GC (Agilent Technologies Inc., Santa Clara, CA). Agilent J & W HP-5MS column, 30 m, 0.32 mm, 0.25 μm was used with helium (purity 99.99%) as a carrier gas (1.0 mL/min). Operating conditions were as follows: oven temperature 60 °C (3 min), 1 °C/min to 80 °C (3 min); 5 °C/min 280 °C (5 min); flow rate of 1 mL/min (He); injector T = 260 °C; FID T = 270 °C; 1-μL injection volume at split ratio 25:1. The MS conditions were: ionization voltage 70 eV, ion source temperature 230 °C, transfer line temperature 280 °C, solvent delay 4.25 min and mass range: 50–550 Da. The MS was operated in scan mode. 1 μL of EO diluted with *n*-hexane (10%, v/v) was injected into the GC/MS system. The GC analysis was carried out using an Agilent 7890A GC system. In order to obtain the same elution order with GC/MS, simultaneous triplicate injections were done by using the same column and same operational conditions.

Identification of the components present in the EO samples was made by comparing mass spectra of components in the EOs with those from National Institute of Standards and Technology (NIST 08) and Adams mass spectra libraries (Adams, 2007), by AMDIS (Automated Mass Spectral Deconvolution and Identification System) and by comparing the literature and estimated Kovat's (retention) indices that were determined using mixtures of homologous series of normal alkanes