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Chemical composition and antimicrobial activity of hydrodistilled oil from juniper berries

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ABSTRACT

This study aimed at investigating the chemical composition and in vitro antimicrobial activity of juniper (*Juniperus communis* L.) berries essential oils (EOs), including commercial samples as well as the oil hydrodistilled from berries grown in Portugal, for which few information is available in the literature. The analysis was performed by gas chromatography coupled to mass spectrometry detection (GC/MS) allowing the identification of a total of 97 compounds. The EOs showed different chemical profiles with only one being according to the European Pharmacopoeia 8 requirements. The laboratory-hydrodistilled EO was characterized by its high content in α-pinene (41.6%), followed by β-pinene (27.6%) and limonene (6.4%), commercial EO1 by α-pinene (31.1%), β-myrcene (16.3%) and sabinene (7.5%) and commercial EO2 by *δ*-cadinene (16.0%), α-pinene (12.2%) and sabinene (9.4%). The distinct chemical profiles were also evidenced by principal components analysis (PCA), with a clear separation of the evaluated EOs. One of the commercial samples, showed the presence of propachlor, a banned herbicide in the European Union. All the EOs showed relevant antimicrobial activity as they presented microbicidal activity against *Candida albicans* and at least six of the ten tested bacteria. Commercial EO2 showed a higher biological activity, as it was active against all tested microorganisms, which could be related to its higher content in sesquiterpenes, in particular those oxygenated. Overall, results support the use of *Juniper communis* L. berries EO as an antiseptic in traditional medicine and highlight its potential as a biopreservative that could be used in different industries.

1. Introduction

Essential oils (EOs) are highly complex mixtures of volatile compounds that are biosynthesized by plants to exert diverse ecological functions, such as acting as defensive substances against microorganisms and herbivores (Bakkali et al., 2008). Since ancient times, EOs have been used in traditional medicine for their various properties including spasmolytic, anti-inflammatory, antioxidant and antimicrobial activities (Lang and Buchbauer, 2012). Additionally, due to their generally pleasant odor and/or flavour, several EOs are currently required in significant amounts by different industries such as cosmetic, perfume, pharmaceutical and food industries (Raut and Karuppayil, 2014).

Recently, consumers are becoming increasingly concerned regarding the use of synthetic preservatives to extend the shelf life of foods and cosmetics. Therefore, there has been a renewed interest regarding the possibility of using plant essential oils as biopreservatives in such products, as some have been shown to possess strong antimicrobial activity against a wide range of bacteria (Burt, 2004; Kunicka-Styczynska et al., 2009; Silva et al., 2013; Sung et al., 2013; Seow et al., 2014). Currently, according to the US Federal Regulation 21CFR182.20, several EO formulations are considered in the category of Generally Recognized as Safe (GRAS) for their intended used, among which juniper oil is included (U.S. Code of Federal Regulations, 2017). Juniper (*Juniperus communis* L.) is a plant worldwide spread belonging to the Cypress family (Cupressaceae) that has been used along the history for many purposes, including in traditional medicine, in gastronomy as a spice and as a natural ingredient in cosmetic, pharmaceutical and food industries. Juniper berries is one of the few spices

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originated from cold climates, being used as a condiment to confer a particular aroma and taste to game meat dishes traditionally cooked in some European regions, such as in northern Scandinavia and in the northeast of Portugal. They are also used in the aromatization of traditional alcoholic beverages and in the production of gin, the most popular juniper-based spirit. According to the European legislation (Council Regulation EEC 1576/89, 1989), the main flavour in the "Distilled gin" class should come from juniper berries. Additionally, since ancient times juniper berries have also been used in folk medicine for its stomachic, diuretic, antiseptic and antirheumatic properties to treat dyspepsia, cystitis, arthritis, gout and other inflammations (Yarnell, 2002; Sela et al., 2011). Its diuretic effect, in particular, has been attributed to the presence of terpinen-4-ol (EMA, 2011; Sela et al., 2011). Juniper berries are inscribed in several Pharmacopoeias, including the 8th edition of the European Pharmacopoeia (Ph. Eur. 8), and are the source of juniper oil, which is also inscribed in the same Pharmacopoeia. The characteristic composition of the essential oil obtained by steam distillation from the ripe, non-fermented berry cones of *Juniperus communis* L. is described on the monograph Iuniperi aetheroleum, which defines the following requirements: 20–50% of α-pinene, 1–35.5% of myrcene, < 20% of sabinene, 2–12% of limonene, 1–12% of β-pinene, < 7% of *trans*-(*E*)-caryophyllene, 0.5–10% of terpinen-4-ol, < 2% of bornyl acetate and < 1% of α-phellandrene. Previous studies carried out on EOs extracted by hydrodistillation from juniper berries of diverse geographical origin, including Greece, Italy, Spain, Serbia, Kosovo, Algeria, Lithuania, Estonia, Macedonia and Slovakia, showed a noteworthy variation both on the qualitative and quantitative profile (Chatzopoulou and Katsiotis, 1993; Falasca et al., 2016; Fejér et al., 2018; Foudil-Cherif and Yassaa, 2012; Glišić et al., 2007; Hajdari et al., 2015; Lo[zbreve]ienė et al., 2010; Orav et al., 2010; Sela et al., 2011; Vichy et al., 2007). While α-pinene was consistently the major compound in most EOs (although presenting a wide variation of content, ranging from 13.4% to 77.4%), a higher variability was found regarding the other compounds present at higher contents. In this regard, the second most abundant compound was most frequently sabinene or β-myrcene, although for some EOs were β-phellandrene, terpinen-4-ol, α-pinene, germacrene D or *δ*-cadinene. In addition, the variability on the chemical composition of the EOs extracted from juniper berries was also evidenced by the fact that several did not comply with Ph. Eur. 8 requirements (Angioni et al., 2003; Chatzopoulou and Katsiotis, 1993; Falasca et al., 2016; Foudil-Cherif and Yassaa, 2012; Lo[zbreve]ienė et al., 2010; Matović et al., 2011; Orav et al., 2010; Vichy et al., 2007). This noteworthy variation among the qualitative and quantitative composition can be ascribed to several factors that are known to influence the chemical composition of plant EOs, such as environmental conditions (climate, soil composition, etc), harvesting period/maturation of the berries and extraction method, among others (Fejér et al., 2018). While several reports can be found in the literature regarding the analysis of the essential oil extracted from juniper berries using a Clevenger type apparatus, few information is found on the chemical composition of commercially available oils (Filipowicz et al., 2003; Höferl et al., 2014; Falasca et al., 2016) Additionally, there is a scarcity of data regarding the chemical composition of juniper EO obtained from wild berries grown in Portugal. Therefore, in this work the chemical composition of three different juniper berries essential oils, namely one obtained on the laboratory by hydrodistillation from juniper berries grown in Portugal and two commercially acquired, was evaluated and compared. Considering that the antimicrobial/antiseptic activity is one of the main bioactive properties described for juniper berries EO, in this work, the antimicrobial activity against several pathogenic and food-spoiling bacteria and one yeast was further assessed, in view of its potential use as a biopreservative.

2. Materials and methods

2.1. Samples

Dried and mature berries of *Juniperus communis* L. were acquired in 2016 from a Portuguese supplier (Alma d'Flor, Almada, Portugal; the berries were collected in the wild, in the center region of Portugal in 2016, according to the supplier). The berries were used for essential oil extraction by hydrodistillation in a Clevenger apparatus in accordance with the description of the European Pharmacopoeia (1996). Briefly, grounded berries (50 g) were placed in a round-bottom flask with 500 mL of distilled water and the mixture was boiled during 3 h. The essential oil was separated from the water and recovered directly without adding any solvent. After being collected, the oil was dried over anhydrous sodium sulfate and stored at −20 °C until being analyzed. Additionally, two commercial essential oils from juniper berries, both labelled as being from *J. communis* berries, were tested in this study, one obtained from the same herbal shop of the berries (Alma d'Flor, Portugal; obtained by hydrodistillation according to the supplier) designated as commercial EO1, and the other from a Portuguese distributor (Dias e Beltrame, Portugal; no information was available regarding extraction method used) designated as commercial EO2.

2.2. GC–MS analysis

The GC–MS unit consisted on a Perkin Elmer Perkin Elmer system (GC Clarus® 580 GC module and Clarus® SQ 8 S MS module) gas chromatograph, equipped with DB-5 MS fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J & W Scientific, Inc.), and interfaced with a Perkin-Elmer Turbomass mass spectrometer (software version 6.1.0, Perkin Elmer, Shelton, CT, USA). Oven temperature was programmed, 45–175 °C, at 3 °C.min^{-1}, subsequently at 15 °C min^{-1} up to 300 °C, and then held isothermal for 10 min; injector temperature, 280 °C and the injection volume of 1 μL. The transfer line temperature was 280 °C; ion source temperature, 220 °C; carrier gas, helium, adjusted to a linear velocity of 30 cm s^{-1}; split ratio, 1:40; ionization energy, 70 eV; scan range, 40–300 u; scan time, 1 s. Identifications were based on the comparison of the obtained spectra with those of the NIST 2011 mass spectral library and were confirmed using linear retention indices determined from the retention times of an n-alkane (C7–C40) mixture analyzed under identical conditions, with comparison with published data (Adams, 2007), and when possible with commercial standard compounds.

Compounds were quantified as area percentages of total volatiles using the relative values directly obtained from peak total ion current (TIC). Analysis were performed in triplicate.

2.3. Antimicrobial activity

The three essential oils were individually tested against 10 different bacterial strains and 1 yeast, namely *Bacillus cereus* (NCTC 10,320), *Bacillus subtilis* (ATCC 6633), *Enterobacter aerogenes* (ATCC 13,048), *Enterococcus faecalis* (ATCC 33,186), *Escherichia coli* (ATCC 10,536), *Klebsiella pneumoniae* (ATCC 13,883), *Proteus mirabilis* (ATCC 14,153), *Pseudomonas aeruginosa* (ATCC 27,853), *Salmonella typhimurium* (ATCC 14,028), *Staphylococcus aureus* (ATCC 29,213) and the yeast *Candida albicans* (ESAB collection). The antimicrobial activity was determined by the broth macrodilution method, based on the methodology described by the Clinical and Laboratorial Standards Institute (CLSI, 2009) with some modifications. Briefly, bacterial suspensions were prepared in Mueller-Hinton broth (MHB) for bacteria or in Yeast Extract Peptone Dextrose broth (YPD) for the yeast, from 24-hour cultures in nutrient agar for bacteria or Sabouraud dextrose agar for the yeast, by adjusting to 0.5 McFarland turbidity standard followed by dilution to approximately 5 × 10^5 CFU/mL. The essential oil was subjected to two-fold serial dilution with MHB added with 0.5% Tween 80 (v/v) in