Therapeutic potentials associated with biological properties of Juniper berry oil (Juniperus communis L.) and its therapeutic use in several diseases – A Review

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ABSTRACT

Juniperus communis L. is a plant that belongs to the Cupressaceae family. It grows as either a shrub or small tree and is widely distributed across the Northern Hemisphere, including northern Europe, Asia, and America. The berries are an efficient source of several bioactive structures. This review article will focus on the current status of the therapeutic use of juniper berry essential oil, which is presently indicated as a herbal medicinal treatment for dyspepsia. Interest in plant-based medicinal products is growing, and therefore it is important that accessible, up-to-date research is available to patients. Many plants are a natural source of therapeutic structures and can therefore often provide an alternative to synthetic pharmacology. A main constituent of juniper berry oil is α-pinene, a highly active structure which has been shown in in vitro and in vivo studies to possess several biological activities. This review sums up the available
reports and indications which describe the function and value of juniper berry essential oil and especially, the constituent α-pinene as a potential candidate in several disorders and inflammatory conditions.

**Keywords:** *Juniperus communis*, dyspepsia, juniper berry oil, Antioxidant activity, Antibacterial activity, Anti-inflammatory activity

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**INTRODUCTION:** Naturally occurring compounds have always been an essential source of therapeutic compounds, both directly and indirectly. The range of ailments treated by natural remedies can vary from mild stress or mood disorders to more severe asthma conditions and digestive problems [1-2]. The process of extracting different structures from plants and employing them in chemical synthesis has been a distinctive approach in drug discovery as well [3-4]. Furthermore, it has been demonstrated that the complex mixture of essential oils (EOs) synthesized by plants play an important role in the defense mechanisms against microorganisms and herbivores [5-6]. Herbal medicines, including essential oils have been applied in various biological conditions since ancient times due to their spasmytic, anti-inflammatory, antioxidant, and
antimicrobial activities [6-7]. Moreover, the usage of EOs is increasing in industrial areas such as cosmetic, perfume, pharmaceutical and food industries [6-9]. After years of predominantly synthetic preservatives for foods and cosmetics or the usage of synthetic drugs, consumers and patients are becoming more concerned about the ingredients of these products. In the same token, a renewing interest in plant-based essential oils as preservatives or treatment has been reported in the population [6], [10-14]. Juniper oil is one possible candidate, as it is categorized as Generally Recognized as Safe (GRAS) by the US Federal Regulation (U.S. Code of Federal Regulations, 2017). Juniperus communis L. is an example of a promising medicinal plant due to its high content of pharmacologically valuable essential oils (EOs). Of particular interest is α-pinene from the group of monoterpenes, which was categorized as a “molecule of interest” due to its biological activities, its therapeutic potential [15], and the high amount found in essential oils gained from Juniperus species [15-16]. This review aims to provide an overview of the evidence for the biological and potential therapeutic effects of Juniperus communis L. essential oil. Literature was searched employing PubMed (http://www.pubmed.ncbi.nlm.nih.gov), ScienceDirect (http://www.sciencedirect.com), Scientific Electronic Library Online (SciELO) (http://www.scielo.org) and Google Scholar (http://www.googlescholar.com). Publications from between 1998 and 2021 included over 20 in vitro and in vivo studies highlighting the pharmaceutical potential of juniper berry essential oil.

While the historical use of juniper in a therapeutic context has been thoroughly reviewed by previous published works such as Al-Snafi (2018) [17], the current review aims to highlight the potential of juniper berry essential oil to recent studies for drug discovery and for therapeutically application.

**Plant background and uses:** Juniperus communis L. (Cupressaceae Rich. ex Bartl.) belongs to the genus family of Juniperus L., which includes around 70 species widely spread worldwide. The broad range of its habitat distribution has resulted in diverse ecological adaptations [18]. It grows over an extensive area in the cold Northern Hemisphere, including the northern regions of Europe, Asia, and North America (Figure 1) [19]. In addition to its use in the pharmaceutical industry, juniper berries are also used as a spice or as a natural ingredient in the cosmetics and food industries, especially in the traditional production of gin [6]. In traditional medicine, the berries were associated with stomachic, diuretic, antiseptic and anti-rheumatic properties and have been applied in the treatment of various inflammatory diseases [6], [20-21].
Chemical composition: Juniper oil, which is described in several Pharmacopoeias (e.g. Ph. Eur. 8, [6]), is typically extracted from juniper berries. The Berries are carefully collected to ensure a harvest of high quality [22]. The volatile compounds are then extracted from the aromatic berries via steam distillation, as this shows the highest efficiency among available methods [23]. These compounds include α-pinene, β-myrcene, sabinene, and δ-limonene as the most significant and are identified through GC/FID and GC/MS methods (Figure 2) [24].

Reported separation methods by studies mentioned in this review differ between authors. According to the European Pharmacopoeia 10th Edition (Ph. Eur. 10), the colorless or yellowish juniper berry essential oil is identified through GC/FID method, using helium as the carrier gas and fused silica as the column material. The composition of the oil is specified as being: 20-50% α-pinene, <20% sabinene, 2-12% limonene, 1-12% β-pinene, 1-35% β-myrcene, <1% α-phellandrene, 0.5-10% terpinen-4-ol, <2% bornyl acetate and <7% β-caryophyllene. Among the different monoterpen hydrocarbons, α-pinene is considered the predominant active component of J. communis [23], [25]. In fact, antibacterial, antifungal, neuroprotective, gastroprotective, and several other biological activities have been shown to be related to the presence of α-pinene in juniper species, which was termed "molecule of interest" [15]. Moreover, α-pinene has shown anti-inflammatory, spasmolytic, antitumor, and other pharmacological effects [15], [26].

Biological activities: There is extensive research available regarding the non-clinical and pre-clinical potentials of juniper berry oil.

Antioxidant additive in dry fermented sausages: A former study demonstrated the activity of J. communis essential oil (JEO) in inhibiting lipid oxidation and bacterial growth of cooked pork sausages. The following research was carried out to investigate the antioxidant
behavior in dry fermented sausages. Results obtained encourage further consideration of utilizing JEO as a healthier substitute for Sodium Nitrite in dry fermented sausages [27].

**Water disinfectant:** An *in vitro* study demonstrated JEO’s role in suppressing biofilm development of *Mycobacterium avium*, and which proposed utilizing it in sterilized natural water as an effective disinfectant [28].

**Antioxidant activity:** *In vitro* assays were carried out to explore the antioxidant activity of aqueous and ethanolic extracts of juniper berries. Results confirm the strong ability of both extracts to scavenge superoxide and hydrogen peroxide radicals, as well as the presence of a metal chelating activity [29]. Following these results, *in vitro* and *in vivo* analyses were conducted on juniper essential oil, showing antioxidant behaviors that correlated with the concentrations utilized [30]. Moreover, *Caenorhabditis elegans* worms were employed to discover the anti-aging effects and antioxidant potential of JEO. The *in vivo* assay revealed a higher survival ratio in worms treated with JEO, in comparison to the control [31].

**Antifungal activity:** *J. communis* berry oil has been investigated for antifungal activity and studies have confirmed synergistic effects between the essential oil components against some tested fungi [32]. The antifungal potential was adapted for extended *in vivo* analysis against dermatophytes yeasts. Subsequently, the essential oil of *J. communis* has shown the desired efficiency as an antifungal agent [33].

**Antibacterial activity:** Approximately 25000 patients in Europe die each year due to multidrug-resistant bacteria infections [34]. Selection among bacteria for anti-bacterial resistance is accelerated by the overuse of antibiotics. Therefore, it is critical that alternatives to antibiotic treatment are identified. Different compounds of the JEO have previously displayed bacterial growth inhibition of *S. aureus* (gram-positive) and *A. baumannii* (gram-negative) [32]. Another assay confirmed antibacterial activity against *S. aureus*, in addition to *E. coli* bacterium [35]. A specific mechanism of antibacterial action was earlier identified as biofilm formation limiting activity. This study was conducted on *Campylobacter jejuni* (*C. jejuni*), revealing the ability of JEO to control and suppress the spread of campylobacters, especially in the food industry [36]. Furthermore, specifically α-pinene was under investigation to study the modulation of antibiotic resistance in *C. jejuni* [34], [37].

**Anti-inflammatory activity:** Juniper oil is known to exhibit anti-inflammatory activity, although several mechanisms for this action have been proposed. Studies suggest that the proficiency of JEO in inhibiting the formation of pro-inflammatory cytokines reduces inflammation [35]. In addition, it has been shown in studies by Rufino et al. (2014) that *Juniperus* essential oil and its major component α-pinene is able to prevent pro-inflammatory pathway activation upon IL-1β-stimulation [34], [38]. Moreover, studies by Kim et al. (2015) demonstrated the effect of α-pinene to reduce pro-inflammatory cytokine levels of IL-6 and TNF-α in macrophages [34], [39]. A different study confirms this anti-inflammatory activity of JEO through *in vivo* discoveries in inflammatory settings of human skin cells. The results obtained suggest therapeutic anti-inflammatory benefits of *J. communis* essential oil within dermal fibroblasts [40].
Renal effects: Diuretic effects of juniper berry have been explored and were attributed to hydrophilic structures present in the fruit [41]. However, other studies suggest that the urine yield increase does not correspond to electrolytes deficiency when utilizing aqueous solutions of juniper berries [42].

Gastrointestinal effects: Shifting the focus to Gastrointestinal (GI) diseases, dyspepsia is a therapeutic indication of juniper berry oil, according to the Committee on Herbal Medicinal Products (HMPC) assessment report in 2010. Furthermore, owing to its anti-spasmodic, carminative, antibacterial, and digestive properties, juniper has been reported to treat several other GI disorders [42]. Additionally, an in vivo study performed on rats confirmed elevated healing effects of gastric ulcerations when applying Juniperus communis extracts. Nevertheless, peptic activity and the pH of the stomach were not affected. Further applications include Ascites, Irritable Bowel Syndrome (IBS), and Hemorrhoids. Effectivity here is believed to be due to diuretic, carminative and mild astringent activities of JEO [43].

Table 1. Biological activity of Juniper berry essential oil and its dominant constituent α-pinene.

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<th>Biological activity</th>
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**In vitro and in vivo analyses on Juniper essential oil, proving different antioxidant behaviours**

[30]

**Caenorhabditis elegans worms were employed to discover the anti-aging effects of Juniper essential oil**

[31]

**Antifungal activity**

This study estimated the antibacterial and antifungal activity of three different Juniper berry oils and their main components. All the micro-organisms used in this experiment were isolated from patients of Regional Hospital of Gdańsk and some of them showed resistance against commonly used antibiotics.

[32]

Minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) were used to evaluate the antifungal activity of the oil against dermatophytes (*Epidermophyton floccosum, Microsporum canis, M. gypseum, Trichophyton mentagrophytes, T. mentagrophytes var. interdigitale, T. rubrum, T. verrucosum*), yeasts (*Candida albicans, C. guillermondii, C. krusei, C. parapsilosis, C. tropicalis, Cryptococcus neoformans*) and Aspergillus species (*Aspergillus flavus, A. fumigatus, A. niger*).

[33]

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[36]

**Anti-inflammatory activity**

Proficiency of Juniper essential oil in inhibiting the formation of pro-inflammatory cytokines

[35]

*In vivo* discoveries in inflammatory settings of human skin cells

[40]

**Renal effects**

Time-dependent diuretic response in rats treated with Juniper berry preparations

[41]

Juniper increases urine output without loss of electrolytes

[42]

**Gastrintestinal effects**

Juniper has been recorded to treat several gastrointestinal disorders

[42]

*In vivo* study performed on rats confirmed elevated healing effects of gastric ulcerations when applying *Juniperus communis* extracts

[43]

**Antitumor/Cytotoxic effects**
DISCUSSION: The biological and potential therapeutic effects of Juniperus communis L. oil have been utilized since the time of ancient medicine, but the renewal of patient desire for natural alternatives to synthetic pharmacology, especially in the face of growing bacterial resistance to antibiotics, has rekindled interest in the oil. The essential oil obtained by the steam distillation follows different production protocols depending on geographical origin, climate, harvesting period or extraction method [6], [50] leading to variation in qualitative and quantitative profile [6], [20], [24], [50-57]. Although showing a wide variation ranging from 13.4% to 77.4%, α-pinene was demonstrated as the consistent major compound [6]. In like manner, it is also important to address misleading information concerning the side effects of this plant. In previous years, Juniperus communis was alleged to cause kidney irritation. However, actual evidence on this claim was never provided. As a matter of fact, recent research published in 2019 has proven the opposite. The study was conducted on diabetic rat models, measuring oxidative stress and kidney function parameters throughout the experiment. The results obtained confirm enhanced kidney function associated with protective effects and promoted antioxidative activity [58], leading to nephroprotection instead of nephrotoxicity. The other side effects described in the literature, address the use of JEO during pregnancy. It is said that the volatile oil of Juniperus communis could act as a gastrointestinal irritant, which might stimulate the uterus during pregnancy and explain why JEO should not be used during pregnancy [59].

With increased demand of natural compounds regarding various of pharmacological properties, this increasing demand represents a risk for high quality [15], [60]. Previous reports demonstrated adulterations of essential oils, therefore in the future quality checks of essential oils are necessary [15], [61-64].

Nevertheless, future clinical trials should emphasize juniper essential oils or its ingredient, α-pinene as a potential therapeutic product closing the gap between pre-clinical study successes and therapeutic usage.
CONCLUSION: In this short review, the importance of essential oils from juniper berries and its major ingredient α-pinene was highlighted. Juniper berry essential oil exhibited various promising biological effects and should be considered for further therapeutic applications within the pharmaceutical industry. For instance, JEO could be extra involved in skincare formulations as a reliable herbal medicine regarding antiseptic, antimicrobial and anti-aging properties.

Encouraged by the various biological and pharmacological potential activities of *Juniperus communis*, carrying out clinical studies for further development is highly appealing. Commercially available juniper berry oil is marketed as Roleca®, which could be the main contributor to future clinical trials conducted within Europe and particularly in Germany.

**List of abbreviations used:** EO: essential oil; JEO: *Juniperus communis* essential oil

**Competing Interests:** The authors have no financial interests or conflicts of interest.

**Authors’ contribution:** All authors contributed to this manuscript.

**Acknowledgement:** With respect to the topic of *Juniper communis* as a functional food, we like to provide the information of functional foods being natural or processed foods that contain biologically active compounds with effective, but non-toxic amounts and documented benefits in health.

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