EVALUATION OF THE ANTIBACTERIAL AND ANTIFUNGAL EFFECT OF *JUNIPERUS COMMUNIS* L. ESSENTIAL OIL

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Abstract. *The present study aimed to evaluate the antimicrobial properties of Juniperus communis L. essential oil against various pathogenic bacteria and fungi, offering new insights into potential approaches to combat infectious diseases, particularly in light of the growing global issue of antibiotic resistance. The microorganisms included in this study were Aspergillus niger, Bacillus cereus, Candida albicans, Escherichia coli, Micrococcus luteus (syn. Sarcina lutea), and Staphylococcus aureus. The antimicrobial activity was assessed using the agar diffusion method, with juniper essential oil (lot 232413, purchased from doTerra International) as the test substance. Ampicillin was used as the control for bacterial strains, while nystatin served as the control for fungal strains. The effectiveness of the treatments was determined by measuring the diameters of the inhibition zones, reported in millimeters. The findings revealed that juniper essential oil exhibited a strong antifungal effect on Aspergillus niger (20.33 mm) and notable antibacterial activity against Micrococcus luteus (22.66 mm) and Staphylococcus aureus (17.33 mm). However, it was less effective against gram-negative bacteria like Escherichia coli (7 mm), which also showed resistance to ampicillin. Additionally, Candida albicans demonstrated complete resistance to both the essential oil and the antifungal nystatin (0 mm). In conclusion, juniper essential oil shows promising antibacterial and antifungal potential, although its efficacy depends on the specific microorganisms tested.*

Keywords: *essential oil, Juniperus communis, antibacterial effect, antifungal effect, antibiotic resistance*

INTRODUCTION

In the current context of medicine, antibiotic resistance has become one of the biggest challenges, globally speaking, with a significant impact on public health and the effectiveness of medical treatments. The World Health Organization (WHO) warns that antibiotic resistance is a growing threat, estimating that by 2050, drug-resistant infections could cause 10 million deaths annually unless appropriate measures are taken to combat it (O'NEILL, 2016). Therefore, there is a strong interest in exploring new approaches to address the challenges associated with this topic.

Current research highlights the potential of plant extracts to eliminate pathogenic microorganisms. Among these, essential oils stand out as one of the most widely used forms of plant-derived extracts. These volatile compounds, derived from plants, are well-known for their antimicrobial, anti-inflammatory, and antioxidant properties (BEZERRA *et al*., 2023; BUTTA *et al*., 2023).

Juniper (*Juniperus communis* L.) is a small, evergreen shrub or tree native to Europe, South Asia and North America (IMBREA, 2016). Widely used since ancient times, this shrub has been valued in herbal medicine for its antidiarrheal, anti-inflammatory, astringent and antiseptic properties, and is also effective in the treatment of various abdominal disorders. The main chemical compounds identified in *J. communis* include alpha-pinene, beta-pinene, apigenin, sabinene, β-sitosterol, campesterol, limonene and cupressuflavone (GONÇALVES *et al*., 2022)

Among the bacteria with pathogenic potential are: *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus* (syn. *Sarcina lutea*) and *Bacillus cereus*. *Escherichia coli* is often implicated in urinary tract and gastrointestinal infections, and *Staphylococcus aureus* is known for its ability to cause a wide range of infections, from skin infections to pneumonia and septicemia. *Micrococcus luteus* has been reported in some human infections, and *Bacillus cereus* can cause infections and food poisoning, particularly in people with compromised immune

systems (DOROBĂȚ, 2006; SĂCĂREA, 2006; MURRAY *et al*., 2015).

Pathogenic fungi that pose a risk to human health include *Aspergillus niger* and *Candida albicans. Aspergillus niger* is a pathogen frequently associated with respiratory infections, while *Candida albicans* is an opportunistic fungus that can cause candidiasis, with infections ranging from
simple mucosal infections to systemic forms that arise in cases of immunosuppression, which are particularly difficult to treat (MĂTĂSĂREAN et al., 2017; DAGENAIS and KELLER, 2009).

MATERIAL AND METHODS

The microorganisms used in this study were: *Staphylococcus aureus (S. aureus), Micrococcus luteus* (*M. luteus) (syn. Sarcina lutea), Escherichia coli (E. coli), Bacillus cereus (B. cereus), Candida albicans (C. albicans), Aspergillus niger (A. niger).* The listed microorganisms were obtained from a medical clinic in Timisoara (except *A. niger*).

Müller-Hinton nutrient media for bacteria and Sabouraud Dextrose Agar for fungi were used for the study. After the process of preparation, pouring into Petri dishes, cooling and solidification of the culture medium was completed, the following step involved inoculating the young microbial suspension. The young fungal suspension of *A. niger* was obtained from cultures. For inoculation, the *"inoculum dissemination method"* was used, which consists of depositing the microbial suspension in an amount of 1 mL/ nutrient medium in a Petri dish (DAGENAIS and KELLER, 2009; MATTEI *et al*., 2014).

Antimicrobial activity was tested by the *"agar diffusion method*". For the application of the method, the next step was to form a well (d = 6 mm) in the nutrient medium of each inoculated plate (SCHWALBE *et al*., 2007). In each well, 0.3 ml of juniper essential oil of 100 percent concentration was deposited. An antibiotic or antifungal biodisc was also applied in the Petri dish as a control. Ampicillin was used for bacteria and nystatin for fungi. For oil diffusion in the medium, the plates were kept 1 hour in the refrigerator. Each microbial species was tested in three replicates. Incubation of the plates inoculated with bacteria was carried out at 37°C for 24 hours and at 25°C for fungal cultures. Antimicrobial activity was determined by measuring the diameter (mm) of each zone of inhibition (HUSSAIN and AL-BAYATI 2022).

The antimicrobial studies of *Juniperus communis* L**.** oil were conducted in the Microbiology Laboratory of the University of Life Sciences "King Mihai I" from Timișoara.

RESULTS AND DISCUSSION

The essential oil used, extracted from the pseudo-berries of *Juniperus communis*, was purchased from doTERRA International, lot 232413 from August 29, 2023. The main chemical components identified in juniper essential oil from doTERRA by GC/MS include: alpha-pinene (38.43%), myrcene (15.61%), sabinene (6.95%), limonene (3.96%), terpinene-4-ol (3.56%), gamma-terpinene (3.42%), and beta-pinene (2.53%). The chemical analysis process was: Gas Chromatography (GC), Mass Spectrometry (MS) (https://www.sourcetoyou.com)

In the current research, *Aspergillus niger* exhibited sensitivity to *Juniperus communis essential oil*, which generated an inhibition zone with a diameter of 20.33 mm. In comparison, nystatin used as a control resulted in an inhibition zone of 30 mm.

The results obtained in the present study are in agreement with the literature data, which emphasize the antifungal effect of juniper essential oil and various fractions and extracts of *Juniperus communis* on species of the genus *Aspergillus*. GLISIC *et al*., (2007) investigated the effect of fractions of juniper essential oil extracted from pseudo-berries. The fractions F2 (63.1% alpha-pinene, 35.7% sabinene) and F3 (21.9% alpha-pinene, 74.9% sabinene) showed an inhibition zone diameter of 20 mm. These results are similar to those observed in our study, thus supporting the sensitivity of *Aspergillus niger* to *Juniperus communis* oil. CAVALEIRO *et al*., (2006) evaluated the activity of the essential oil extracted from *Juniperus communis* (ssp. *alpina*) pseudo-berries and determined a minimum inhibitory concentration (MIC) of 10-20 µL/mL and a minimum lethal concentration (MLC) of >20 µL/mL against *Aspergillus niger*. The MIC of 10-20 µL/mL suggests that juniper essential oil is effective in inhibiting the growth of *Aspergillus*

niger at moderate concentrations, whereas an MLC of >20 µL/mL indicates that a higher concentration is required for full antifungal activity.

Regarding the hydroalcoholic extracts from *Juniperus communis* pseudo-berries, the study by ASILI *et al*. (2008) reported significant antifungal activity at a concentration of 200 mg/mL against *Aspergillus niger*, with an inhibition zone of 20.9 mm. Thus, a similarity can be observed between the values obtained for the hydroalcoholic extracts and those recorded in our experiment with the essential oil. Concerning the aqueous extracts from *Juniperus communis*, DZIEDZINSKI *et al*., (2020) noted that these exhibit a reduced inhibitory activity against filamentous fungi (6 mm for the *Aspergillus* genus and 2 mm for the *Fusarium* genus). Thus, the effect of the essential oil utilized in our study was found to be superior in comparison to the aqueous extracts.

Candida albicans did not show any antifungal effect from the essential oil of *Juniperus communis* in the present study, with an average inhibition zone value of 0 mm. Additionally, the microorganism exhibited resistance to nystatin (used as a control), which also had a value of 0 mm

A separate investigation also shows that the *Juniperus communis* essential oil extracted from leaves had no inhibitory effect (ASILI *et al*., 2008). Also, PEPELJNJAK *et al*., (2005), analyzing the effects of a juniper essential oil extracted from pseudo-berries, obtained a slight inhibitory effect, the diameter of the inhibition zone being 9 mm. The composition of juniper essential oil, however, is different from the composition of the essential oil used in our study. In the case of the essential oil from our own research, the percentage of alpha-pinene is higher by about 10%, and also the concentration of myrcene is higher by about 15%. Meanwhile, sabinene is about 6% lower and beta-pinene 15.31% lower than in the essential oil from the comparative study. It can be observed that the compositional structure of essential oil influences the level of inhibition. In addition, GLISIC *et al*., (2007), analyzed the antimicrobial action of different fractions of juniper oil extracted from pseudo-berries. Thus, in the case of the fungus *Candida albicans*, at high concentrations of 99.5% alpha-pinene, a diameter of the zone of inhibition of 17.00 mm was recorded, this value being the highest, followed by the concentration of 63.1% alpha-pinene and 357% sabinene with zone of inhibition of 11.00 mm. At low concentrations of alpha-pinene, lower values for the zones of inhibition were obtained. Thus, it is found that one of the most important bioactive products required for good inhibition is alpha-pinene. Likewise, CAVALEIRO *et al*., (2006) evaluated the activity of essential oil extracted from the pseudo-berries of *Juniperus communis* ssp. *alpina* from Portugal, demonstrating an inhibitory action on the fungus *Candida albicans* at a minimum inhibitory concentration of 5-10 µL/ml, as well as at a minimum concentration of 20 µL/ml. Thus, these results suggest that the essential oil of juniper from this subspecies has a sensitizing effect on the analyzed species.

Likewise, KUMAR *et al*., (2010), analyzing hydroalcoholic extracts from *Juniperus communis* leaves, observed significant antifungal activity against *Candida albicans* compared to the essential oil tested in this study. Specifically, the methanol extract recorded an inhibition zone of 16.00 mm ± 0.5 mm, while the ethanol extract recorded 19.00 mm ± 1.2 mm. Furthermore, compounds from juniper leaves extracted using petroleum ether and chloroform exhibited relatively high inhibition zones of 16.5 ± 1 mm and 22.00 ± 0.5 mm, respectively. In contrast, the aqueous extracts from juniper leaves in the same study showed no inhibitory effects.

Testing of juniper essential oil extracted from pseudo-berries in the current study demonstrated antibacterial activity against *Bacillus cereus*, with an average inhibition zone diameter of 10 mm. The mean inhibition zone diameter for the ampicillin control was 24.33 mm.

Comparing their findings with other studies, PEPELJNJAK et al., (2005) obtained results similar to those in this study using essential oil extracted from dried pseudo-berries of *Juniperus communis* from Croatia, where the mean inhibition zone diameter was 16 mm.

COSENTINO *et al*., (2003) reported that essential oils extracted from the pseudo-berries of *Juniperus communis* from Sardinia exhibited moderate effects on *Bacillus cereus*, particularly in comparison to the Gram-negative bacteria tested, which demonstrated resistance. This observation aligns with our findings, where certain Gram-positive bacterial strains exhibited greater susceptibility than the Gram-negative bacteria tested, specifically *E. coli*. GLISIC *et al*.,

(2007) demonstrated that at low concentrations (20 μL), juniper essential oil extracted from pseudo-berries harvested in October from Bosnia and Herzegovina showed no susceptibility effect against *Bacillus cereus*. However, at a moderate concentration (100 μL), the same oil demonstrated notable bactericidal action against *Bacillus cereus*, with a zone of inhibition of 15 mm ± 0.10 mm. Thus, it can be seen that the concentration level of the essential oil used directly influences the antibacterial effect.

In addition, MËRTIRI *et al*., (2024) demonstrated that hydroalcoholic extracts from the leaves and pseudo-berries of *Juniperus communis*, harvested from northern Albania in winter, also exhibited good bactericidal action on some *Bacillus* species. Slight differences in inhibition zone diameters (expressed in mm) were observed between leaf and pseudo-berries extracts, with inhibition zone diameters of 23.5 ± 2.12 mm for leaf extracts and 25.75 ± 3.89 mm for pseudo-berries extracts. In both cases, the hydroalcoholic extracts exhibited inhibition zones greater than the control, represented by chloramphenicol (22.00 ± 1.41 mm), as well as the solvent used in the extraction.

The species *Escherichia coli* did not show a significant inhibitory response to *Juniperus communis* essential oil in the present study, recording an average inhibition zone size of 7 mm. This value was greater than that of ampicillin, which showed absolute resistance with a diameter of 0 mm.

Previous investigations indicate that *E. coli* was resistant to the action of juniper essential oil (KALABA *et al*., 2020; PEPELJNJAK *et al*., 2005).

Also, GLISIC *et al*., (2006) analyzed the action of different fractions of an essential oil of *Juniperus communis* from pseudo-berries, on *E. coli ATCC 8739*. The most significant results (16 mm) were obtained in the F1 fraction (99.5 alpha-pinene). It can be seen that the percentage of alpha-pinene most influenced the inhibitory effects of the essential oil on *E. coli.* ROMEO *et al*., (2008), also analyzed the action of an essential oil extracted from *Juniperus communis* pseudo-berries harvested in December. A sensitive action on *Escherichia coli ATCC 25922* was found at all three concentrations used. Thus, at 1 mg/L, 3 mg/L, and 5 mg/L, the diameter of the zone of inhibition was 25 mm, 28 mm, and 39 mm, respectively. It can be seen in this case that the level of inhibition was directly proportional to the concentration of the essential oil used.

It was found that hydroalcoholic extracts from leaves and pseudo-berries of *Juniperus communis* have notable antibacterial action against the pathogenic species *Escherichia coli:* 17.50 ± 0.71 mm (diameter of zone of inhibition) in the case of pseudo-berries extract and 18.50 ± 0.71 mm (diameter of zone of inhibition) in the case of leaf extracts. (MËRTIRI *et al*., 2024) In addition, DIGVIJAY et al., 2017 report that, all crude organic leaf extracts (methanol, ethanol, hexane and chloroform) except aqueous extract possess good antibacterial activity against five pathogenic multi-drug resistant bacteria such as *Escherichia coli*.

In the case of the bacterium *Micrococcus luteus* (syn. *Sarcina lutea*), the effect of *Juniperus communis* essential oil analyzed in the current research was the highest among the microorganisms tested, with a mean inhibition zone value of 22.66 mm across three plates. The control (ampicillin) showed a diameter of 45.66 mm.

In comparison, KALABA *et al*., (2020), observed that, among all Gram-positive bacterial strains, dried pseudo-berries oils obtained by ethanol extraction exhibited the strongest antibacterial activity on *Micrococcus luteus (syn. Sarcina lutea), Bacillus subtilis* and *Bacillus mycoides* species. This result is synergistic with the result obtained in the current research, where the best inhibitory activity of juniper essential oil was observed on *Micrococcus luteus* (syn. *Sarcina lutea*) bacteria.

Staphylococcus aureus showed in a separate investigation an average inhibition zone value of 17.33 mm when exposed to Juniperus communis essential oil, suggesting a notable inhibitory effect. The mean value of the zones of inhibition for the antibiotic ampicillin was 39.33 mm.

Additional research in the field shows potential effects of *Juniperus communis* oil on *Staphylococcus aureus ATCC 6538* (11 mm) and *Staphylococcus aureus MFBF* (14 mm). The main bioactive compounds of this oil were: alpha-pinene =29.17%, beta-pinene=17.84%, sabinene =13.55%, limonene =5.52% and myrcene =0.33% (PEPELJNJAK *et al*., (2005). In this

case, the alpha-pinene content was about 10% lower than the oil used by us, which may explain the lower result compared to the findings of the present investigation. In addition, another study shows that the essential oil of *J. communis* growing spontaneously in Kosovo was determined to exhibit moderate to high activities on *Staphylococcus aureus* and *Hafnia alvei* species, with an inhibition zone of 29 mm for a concentration of 5 mg/ml. However, *Pseudomonas aeruginosa* was resistant to the inhibitory activity of the essential oil. The same study also reports different mean values of the zones of inhibition of the same oil but at different concentrations (the oil was thus diluted with absolute alcohol beforehand). The results obtained with concentrations of 20%, 50% and 100% respectively were better than the results of gentamicin (HAZIRI *et al*., 2013). At the same time, SELA *et al*., (2013) demonstrate in a study in which the activity of an essential oil of *Juniperus communis* (obtained from leaves harvested in late autumn from different areas of Macedonia) was tested, that *Staphylococcus aureus* and *Streptococcus pyogenes* bacteria had the highest MIC (minimum inhibitory concentration), 125 µl/ml, among all 16 microorganisms (Gram-positive, Gram-negative and fungi).

MËRTIRI *et al*., (2024), report that some hydroalcoholic extracts from *Juniperus communis* leaves and pseudo-berries also showed good bactericidal action on Staphylococcus aureus species. At the same time, slight differences were found between the values of the zones of inhibition for the extracts obtained from leaves and those obtained from pseudo-berries: 18.50 ± 0.71 mm and 26.00 ± 1.41 mm, respectively. In both cases, hydroalcoholic extracts did not exceed the chloramphenicol control (30 ± 0.71 mm).

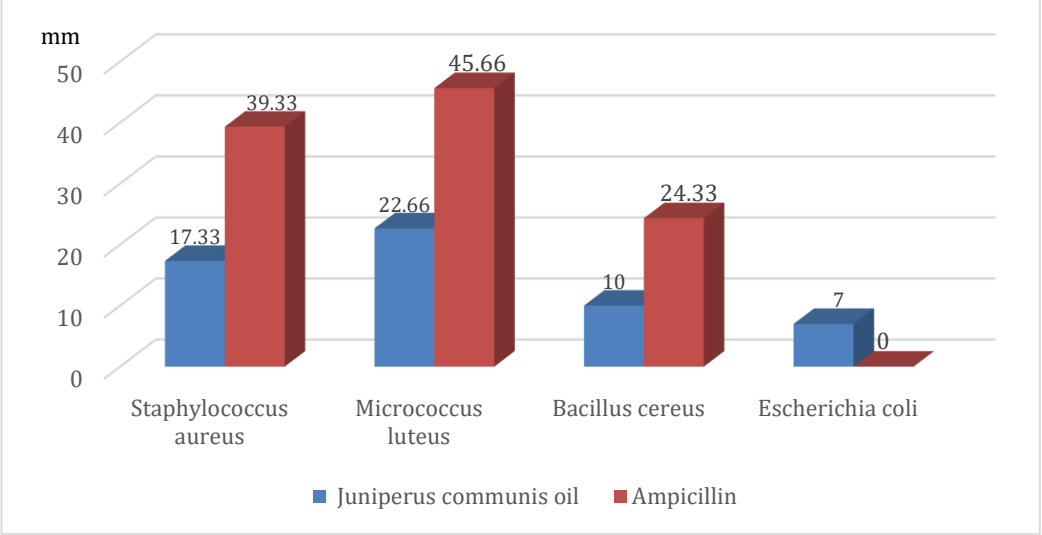

Figure 1. The effect of *Juniperus communis* essential oil and ampicillin on pathogenic bacteria

CONCLUSIONS

The oil of *Juniperus communis* extracted from the pseudo-berries (purchased from doTERRA International), has antifungal and antibacterial effects, but this effect depends on the species analyzed.

The inhibitory effect of juniper oil was strong against *Aspergillus niger* and *Micrococcus luteus* (syn. *Sarcina lutea)*, and it exhibited intermediate to high sensitivity against *Staphylococcus aureus*. Gram-positive bacteria were more susceptible to the essential oil's action compared to the Gram-negative species tested, such as *Escherichia coli*. In our study, the oil of *Juniperus communis* did not show any significant effect on *E. coli*; however, its efficacy was observed to be higher than that of ampicillin.

Candida albicans was found to be resistant to the action of the oil in this study,

suggesting that different concentrations should be tested

The antimicrobial effectiveness of juniper oil is also influenced by factors such as the region of origin, the timing of the plant material harvest, the parts of the plant used for extraction, and the type and chemical composition of the extract. The compound in *Juniperus communis* essential oil with potent antimicrobial activity is alpha-pinene.

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