



UNIVERSITY OF LIFE SCIENCES "KING MIHAI I" FROM TIMISOARA

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"Young people and multidisciplinary
research in applied life sciences"

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

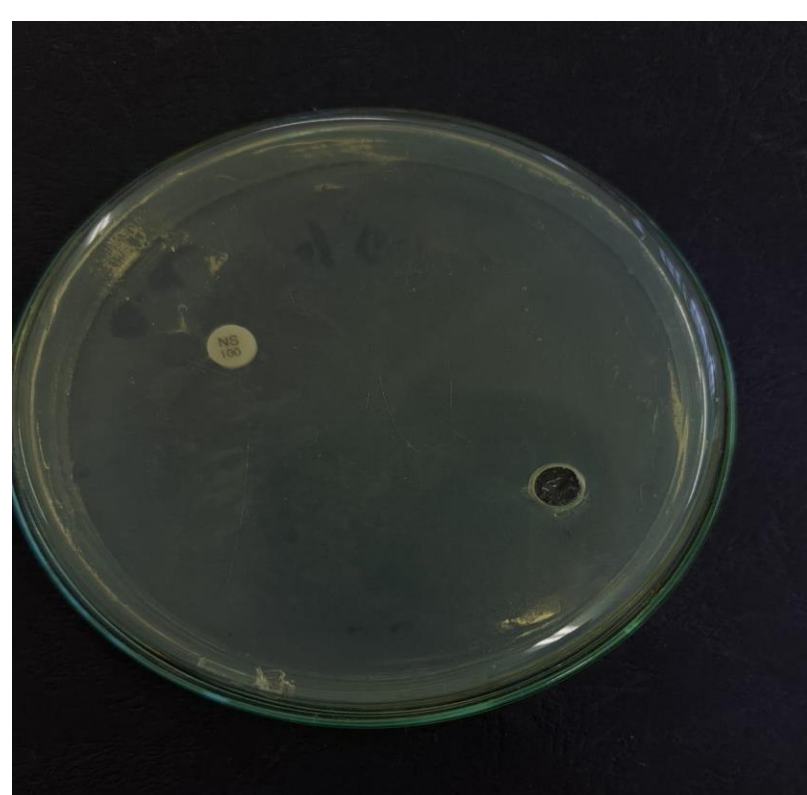
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Abstract: The present study aimed to evaluate the antimicrobial properties of *Juniperus communis* L. essential oil against various pathogenic bacteria and fungi, offering new insights into potential approaches to combat infectious diseases, particularly 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.



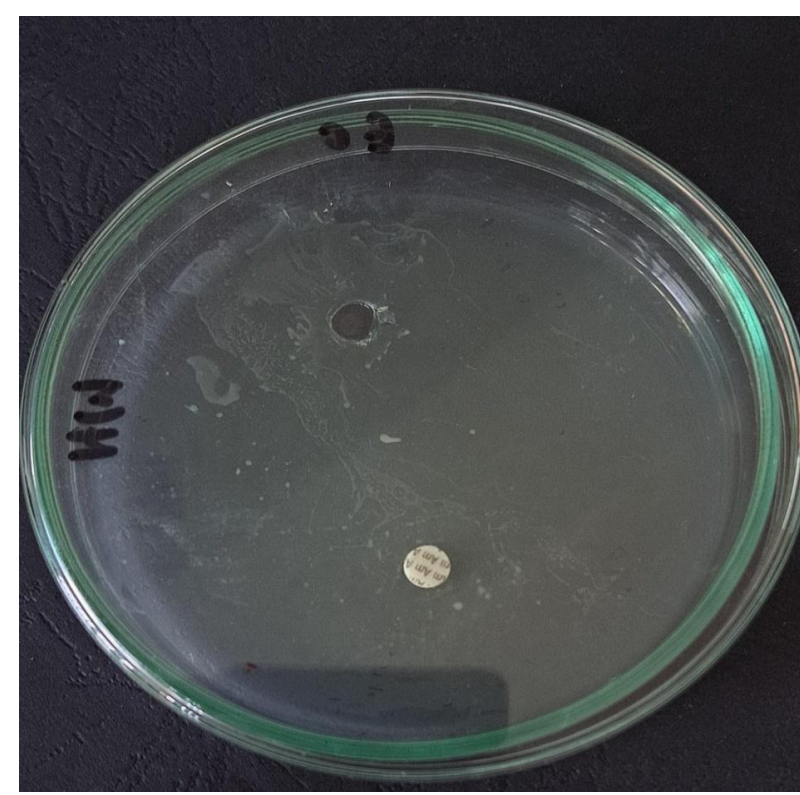
Aspergillus niger



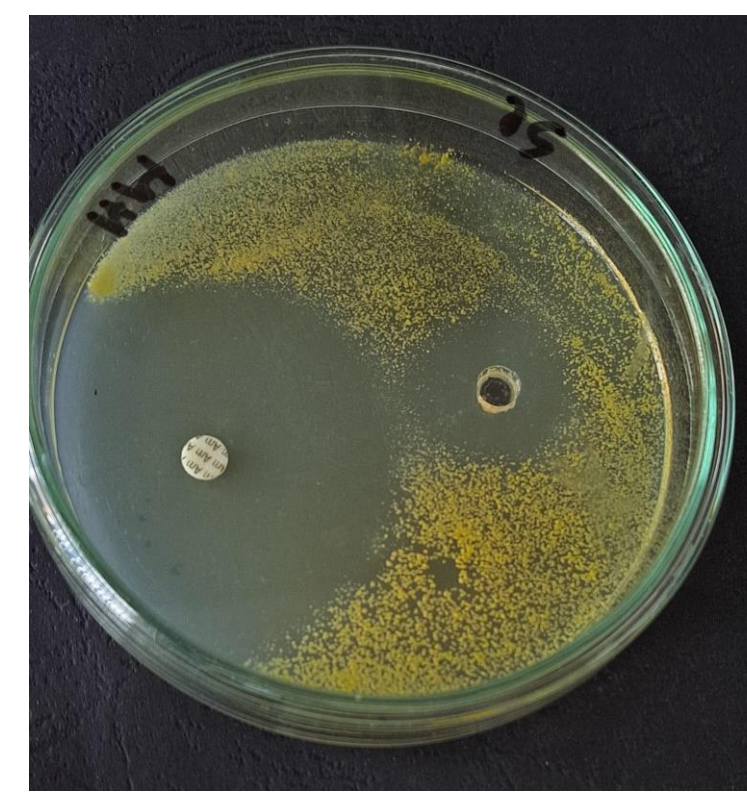
Candida albicans



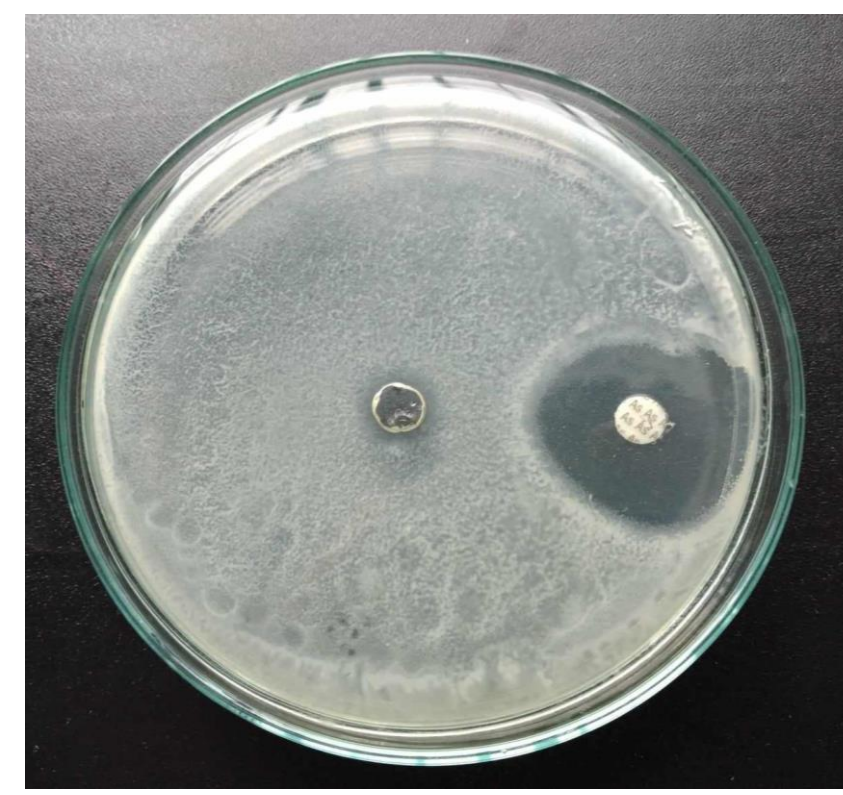
Bacillus cereus



Escherichia coli



Micrococcus luteus (syn. *Sarcina lutea*)



Staphylococcus aureus

• Introduction

In the current context of medicine, antibiotic resistance is one of the most pressing global challenges. For this reason, there is growing interest in exploring new therapeutic strategies to address this complex problem. Bacteria with human pathogenic potential include *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Micrococcus luteus* (syn. *Sarcina lutea*). Also the fungi *Candida albicans* and *Aspergillus niger* possess pathogenicity and antifungal resistance. The use of medicinal plants is currently a promising research direction and is increasingly being investigated for its therapeutic potential in combating microbial resistance. Among the medicinal plants recognized for their antiseptic properties is juniper (*Juniperus communis* L.), a conifer of the Cupressaceae family.

• Material and method

The present study used microorganisms such as *Staphylococcus aureus*, *Micrococcus luteus*, *E. coli*, *Bacillus cereus*, *Candida albicans* and *Aspergillus niger*, obtained from a clinic in Timisoara (except *A. niger*). The oil used, extracted from *Juniperus communis* pseudo-berries, was purchased from doTERRA International, lot 232413 on August 29, 2023. The main chemical constituents identified in doTERRA 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 was chromatography (GC) and mass spectrometry. Müller-Hinton culture media for bacteria and Sabouraud Dextrose Agar for fungi were used for testing. Inoculation was performed by the *inoculum spreading method*, and antimicrobial activity was evaluated by *agar diffusion method* using juniper essential oil at 100% concentration and antibiotic discs (ampicillin for bacteria and nystatin for fungi) as controls. Antimicrobial activity was determined by measuring zones of inhibition. The studies took place at the University of Life Sciences "Regele Mihai I" in Timișoara.

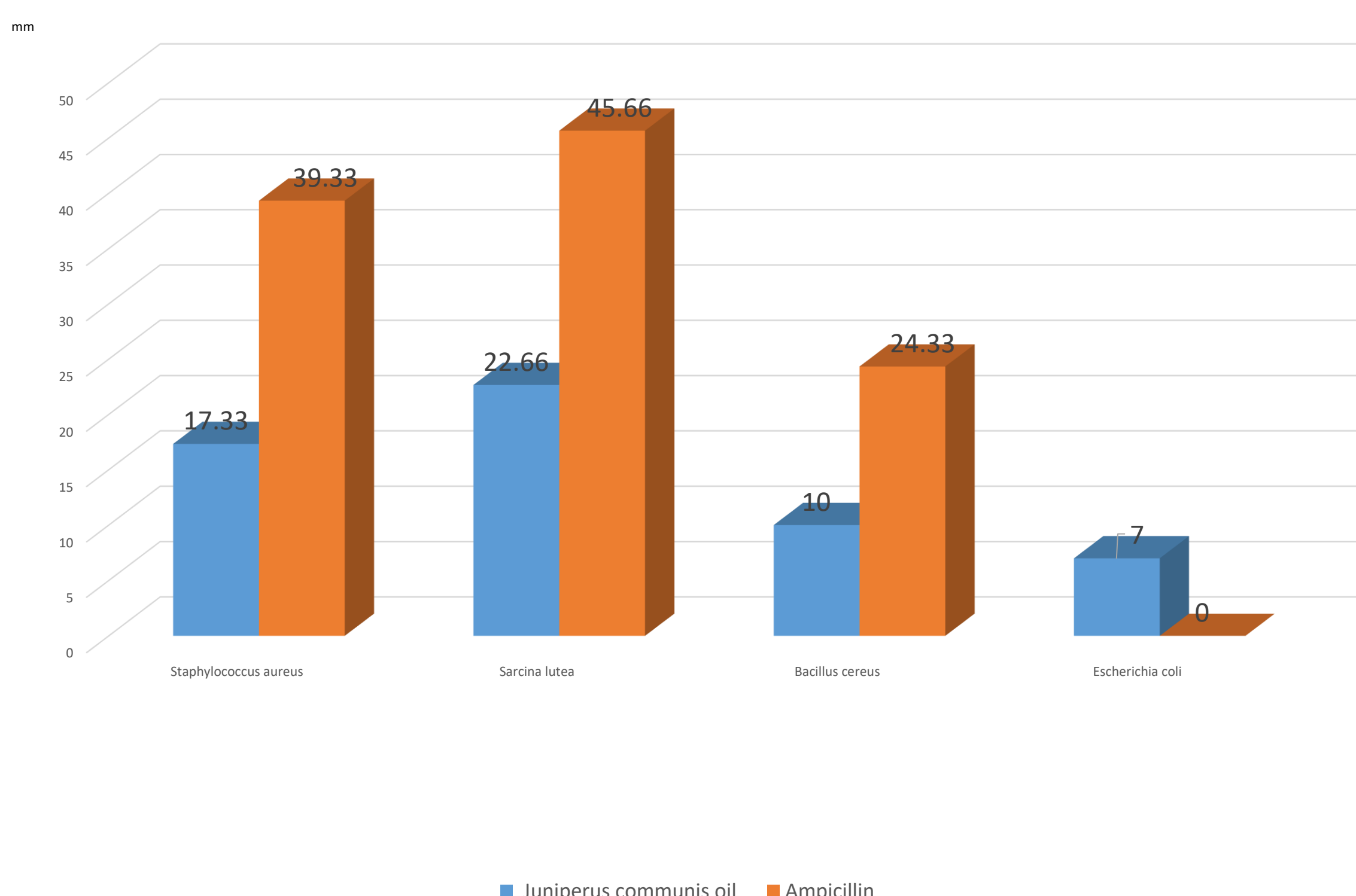


Figure 1. The effect of *Juniperus communis* essential oil and ampicillin on pathogenic bacteria

• Results and discussions

In the present research, *Aspergillus niger* showed sensitivity to *Juniperus communis* essential oil, with a zone of inhibition of 20.33 mm, compared with 30 mm for nystatin, the control used. The results of this study are supported by other research confirming the antifungal effect of juniper oil on *Aspergillus niger*. Glisic *et al.*, (2007) reported an inhibition zone of 20 mm and Asili *et al.*, (2008) obtained a zone of inhibition of 20.9 mm for hydroalcoholic extracts. Cavaleiro *et al.*, (2006) demonstrated the efficacy of juniper oil with a minimum inhibitory concentration (MIC) of 10-20 µL/mL. By comparison, aqueous extracts, according to Dziedzinski *et al.*, (2020), showed a lower inhibitory activity of 6 mm for the genus *Aspergillus*, thus emphasizing the superiority of the essential oil.

The fungal species *Candida albicans* showed no antifungal effect on *Juniperus communis* oil, the zone of inhibition being 0 mm, similar to nystatin (control). Other studies report variations: Asili *et al.*, (2008) observed no inhibitory effect for juniper leaf oil, while Pepeljnjak *et al.*, (2005) obtained a zone of 9 mm for pseudo-berries oil, the differences being attributed to the composition of the oil. At high concentrations of alpha-pinene, Glisic *et al.*, (2007) obtained an inhibition of 17 mm. Other studies (Cavaleiro *et al.*, 2006; Kumar *et al.*, 2010) indicate inhibition of up to 22 mm for alcoholic extracts, emphasizing the variability of the effect depending on the type of extract.

In their own research, juniper oil extracted from pseudo-berries showed an antibacterial effect on *Bacillus cereus*, with a zone of inhibition of 10 mm, compared with ampicillin (24.33 mm). Studies by Pepeljnjak *et al.*, (2005) showed similar results, with a zone of 16 mm using oil from Croatia, and Cosentino *et al.*, (2003) observed moderate effects on *B. cereus* from Sardinian oils. Glisic *et al.*, (2007) found that, at a concentration of 100 µL, juniper oil extracted in October from Bosnia and Herzegovina showed an inhibition zone of 15 mm for *B. cereus*, but no effect at 20 µL. Hydroalcoholic extracts from Albania (Mértiri *et al.*, 2024) had a higher bactericidal action than controls, with zones of 23.50 mm for leaves and 25.75 mm for pseudo-berries.

In the present study, *Escherichia coli* had an inhibition zone of 7 mm to juniper essential oil compared to ampicillin (0 mm). According to other studies, *E. coli* showed resistance to juniper oil (Kalaba *et al.*, 2020; Pepeljnjak *et al.*, 2005), but alpha-pinene rich fractions (Glisic *et al.*, 2006) showed stronger inhibitory effects (16 mm). Romeo *et al.*, (2008) reported that zones of inhibition increase at high concentrations, reaching 39 mm at 5 mg/L. Also, hydroalcoholic (Mértiri *et al.*, 2024) and organic extracts (Digvijay *et al.*, 2017) showed notable antibacterial activity against *E. coli*.

The bacterial species *Micrococcus luteus* had the highest sensitivity to the essential oil of *Juniperus communis*, with a zone of inhibition of 22.66 mm compared to ampicillin (45.66 mm). Similarly, Kalaba *et al.*, (2020) observed that the oils extracted from dried pseudo-berries had potent antibacterial activity on *M. luteus*, *Bacillus subtilis* and *Bacillus mycoides*, thus confirming the effectiveness on Gram-positive strains.

Staphylococcus aureus had a zone of inhibition of 17.33 mm to *Juniperus communis* essential oil, compared to ampicillin (39.33 mm), indicating a notable inhibitory effect. Pepeljnjak *et al.*, (2005) reported inhibitions of 11-14 mm, attributed to a lower alpha-pinene content. Haziri *et al.*, (2013) showed that juniper oil from Kosovo had a stronger effect at higher concentrations (29 mm at 5 mg/ml). Also, Sela *et al.*, (2013) observed a high MIC for *S. aureus* (125 µl/ml). Similarly, for hydroalcoholic extracts, Mértiri *et al.*, (2024) reported zones of inhibition of 18.5 mm for leaf extract and 26 mm for pseudo-berries extract.

• Conclusions

The essential oil of *Juniperus communis*, extracted from pseudo-berries and purchased from doTERRA International, has demonstrated antifungal and antibacterial properties, with varying effects depending on the microorganism. The study showed a strong inhibitory activity against *Aspergillus niger* and *Micrococcus luteus* (syn. *Sarcina lutea*), and an intermediate to high sensitivity for *Staphylococcus aureus*. In comparison, Gram-positive bacteria showed a higher susceptibility to essential oil than *Escherichia coli*, where the effect was low but superior to that of ampicillin. *Candida albicans* proved resistant to the action of the oil, suggesting the need to test varying concentrations. The antimicrobial activity of juniper oil is influenced by factors such as geographical region of harvest, season and parts of the plant used, with the chemical composition of the extract playing a major role, alpha-pinene being identified as the main active compound.