



## Article

# Preliminary Studies on In Vitro Antibacterial Activity Against *Staphylococcus aureus* of Supercritical Fluid Extract from *Juniperus oxycedrus*: Evidence on Phenols Effect

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## Abstract

**Background:** The growing interest in developing new bioactive agents from natural sources led to medicinal and aromatic plants. These plants provide valuable phytochemicals that can serve as natural preservatives, food additives, and flavorings, with various applications. The aim of this study is to evaluate the potential of *Juniperus oxycedrus* berries' supercritical extract through preliminary screenings related to in vitro antibacterial activity, as well as bioinformatics assessments of absorption and toxicity. **Methods:** Supercritical carbon dioxide (CO<sub>2</sub>) was used to extract the bioactive phytochemical compounds from the berries. The extract was characterized using spectrophotometric methods and reverse-phase high-performance liquid chromatography (RP-HPLC). The antibacterial potential was tested against *Staphylococcus aureus* ATCC 25923, where the Minimal Inhibitory Concentration and the Minimal Bactericidal Concentration were determined. Additionally, the influence of the extract on the growth curve kinetics of *S. aureus* was assessed. For the bioinformatics analyses, SwissADME and ProTox-3.0 prediction software were utilized, focusing on the identified phenolic compounds as fingerprint molecules. **Results:** The results demonstrated that exposure to the juniper extract inhibited bacterial growth, resulting in a prolonged lag phase of 6 to 8 h, depending on the concentration of the extract. The software predictions revealed that the investigated phenolic compounds might exhibit high gastrointestinal absorption, along with potential interactions with metabolic mediators and pathways. **Conclusions:** The in vitro and in silico findings support the application of *J. oxycedrus* berries extract as an alternative or complementary strategy for pharmacological treatment and food applications aimed at targeting *S. aureus*.

**Keywords:** medicinal and aromatic plant; green extraction; antioxidant activity; antibacterial activity

Academic Editors: Adriána Liptáková and Iryna Voronkina

Received: 18 January 2026

Revised: 2 February 2026

Accepted: 4 February 2026

Published: 8 February 2026

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## 1. Introduction

*Staphylococcus aureus* is a Gram-positive, facultative anaerobic bacterium with optimal growth conditions of 37 °C and pH 7.4 [1]. It is commonly found in skin and mucosal

microbiota of humans and animals, with 20–30% of the healthy population acting as persistent carriers [2,3]. *S. aureus* is an opportunistic pathogen capable of causing a broad spectrum of infections, ranging from minor skin and soft tissue lesions to severe invasive diseases such as bacteremia, endocarditis, osteomyelitis, and sepsis [4,5]. The pathogenic potential of *S. aureus* is primarily associated with its virulence factors, including pore-forming toxins (hemolysins, Panton–Valentine leukocidin, and phenol-soluble modulins), exfoliative toxins, and superantigens such as enterotoxins and toxic shock syndrome toxin-1 [1,4]. These molecules are able to evade host immune defenses, disrupt cellular integrity, and induce severe systemic conditions such as toxic shock syndrome and staphylococcal food poisoning. The increasing emergence of methicillin-resistant *S. aureus* (MRSA) represents a critical global health concern. MRSA is one of the six ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), recognized for its high adaptability and resistance to  $\beta$ -lactam antibiotics and other antimicrobial classes, leading to persistent infections and therapeutic failures. Overuse of antibiotics in clinical and veterinary settings has accelerated the selection of multidrug-resistant (MDR) *S. aureus* strains, including vancomycin-resistant isolates [3,5].

Other than clinical infections, *S. aureus* poses a significant risk to food safety. It is frequently isolated from high-protein foods such as meat, dairy, and bakery products. Foodborne intoxication caused by staphylococcal enterotoxins, which are heat-stable molecules that remain active even after cooking or pasteurization, are major concern in foodborne outbreaks [2,5]. Inadequate hygiene practices, temperature abuse, and contamination from asymptomatic nasal carriers during food handling further increase the risk of staphylococcal food poisoning [2,6]. According to the *European Union (EU) One Health Zoonoses Report*, published in 2024 from the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), *Staphylococcus* spp. contamination has been reported across diverse food categories in multiple EU Member States, emphasizing the need for enhanced surveillance and preventive strategies [7].

As antibiotic resistance continues to be a significant issue, recent research has focused on alternative antimicrobial strategies. Studies on plant-derived natural products have shown their potential as effective antibacterial agents against *S. aureus*. Compounds such as terpenoids, flavonoids, and organosulfur compounds act by interfering with bacterial cell wall synthesis and can be effective either alone or synergistically with conventional antibiotics. Their broad antibacterial activity, coupled with low toxicity toward mammalian cells, makes these compounds attractive candidates for new therapeutic approaches. The systematic use of natural molecules is strongly encouraged, as it may help address the rising challenge of antimicrobial resistance [2,8]. Several studies have provided empirical evidence of the antimicrobial activity of *Juniperus oxycedrus* L. extract and essential oils (EOs) against various microorganisms, particularly against *S. aureus* and MRSA strains [9–13].

*Juniperus oxycedrus* L. is a medicinal and aromatic plant (MAP) that belongs to the *Juniperus* L. genus of the Cupressaceae family. This small tree or shrub is widely distributed across the Mediterranean region, around the Black Sea, and in the Middle East, and grows in a rocky soil at elevations of up to 1600 m above sea level [9,12,14]. *Juniperus* species contains various phytochemical compounds, including terpenes, flavonoids, phenolic acids, resin, tannins, and organic acids, which provide antimicrobial, antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, insecticidal, and cytotoxic properties [15]. The female cones/galbulus of *Juniperus*, commonly referred to as “fruits” or “berries”, are primarily used as a spice in cuisine, providing a strong and distinctive flavor. In traditional medicine, the berries of *J. oxycedrus* have been utilized to treat common colds, gastrointestinal issues, calcinosis in joints, hemorrhoids, and urinary inflammation. The

berries also serve as an expectorant for coughs, a hypoglycemic agent, and a diuretic for passing kidney stones. Additionally, the berries and leaves can be applied externally to address parasitic diseases [12,14].

Supercritical fluid extraction (SFE) is an efficient and environmentally friendly alternative to traditional extraction methods. In SFE, CO<sub>2</sub> is the most common choice due to its advantageous properties. CO<sub>2</sub> has a low critical temperature of 31.1 °C and pressure of 7.38 MPa, is inert, non-toxic, non-flammable, affordable, and easily removable. This aligns with the global movement towards sustainable “green” extraction methods. Additionally, supercritical plant extraction systems function within a closed-loop configuration, allowing for CO<sub>2</sub> recycling, which provides cost-effectiveness for industrial applications. In the supercritical state, CO<sub>2</sub> diffuses through materials like a gas while simultaneously dissolving compounds like a liquid. This versatility enables the extraction of a wide range of bioactive phytochemical compounds, in particular thermosensitive volatile and non-volatile substances, without compromising their integrity, an issue often encountered in conventional extraction methods. Through precise control of temperature and pressure, SFE facilitates the selective isolation of target compounds based on differences in solubility and polarity [16–19].

Continuing from our previous study involving the same raw material extracted by ultrasound-assisted extraction with acidified hydroethanolic solvent [11], the present research aims to explore the potential of a “green” extraction method suitable for industrial-scale extraction. This preliminary screening focuses on the supercritical extract of *J. oxycedrus* berries, evaluating its in vitro antibacterial activity against *S. aureus* ATCC 25923, given the risks associated with this bacterium. This particular strain is well-characterized, antibiotic-sensitive, and commonly used as a quality control standard in microbiology laboratories for antibiotic susceptibility testing. In addition to improvements in the Minimal Inhibitory Concentration (MIC) and the Minimal Bactericidal Concentration (MBC) values of the *J. oxycedrus* berries supercritical extract (JoB-SFE) compared to our previous study, theoretical and computational investigations were conducted here to predict the absorption and toxicity of the phenolic compounds identified in the extract. This serves as a supporting means for the application of this medicinal and aromatic plant, as an alternative bioactive agent derived from natural sources for controlling and preventing *S. aureus* in therapeutic applications (either alone or in combination with antibiotics) and food safety strategies.

## 2. Results

### 2.1. Extraction Yield, Global and Individual Characterization of Phenolic Compounds

The concentrated extract obtained from the *Juniperus oxycedrus* berries (JoB) exhibited a pronounced aromatic profile and a creamy, semi-solid consistency. Upon refrigeration, the extract formed crystals, suggesting the presence of compounds with low solubility at reduced temperatures. Considering the extraction conditions employed (45 °C and 30 MPa using supercritical CO<sub>2</sub>), these characteristics indicate a complex mixture of both volatile terpenoids and heavier, non-volatile lipophilic components, including waxes, long-chain terpenes, fatty acids, and phytosterols. These substances tend to co-extract under SFE conditions due to the enhanced solvating power of CO<sub>2</sub> at elevated pressures [17].

Table 1 shows the extraction yield and phenolic composition (global and individual) of the JoB-SFE. The extraction yield obtained in our work was 4.35%, which is higher than those typically obtained using conventional techniques. For instance, hydrodistillation of *J. oxycedrus* ssp. *macrocarpa* aerial parts, collected in Sardinia, yielded 0.18% EO [12]. Comparable studies using supercritical CO<sub>2</sub> extraction have reported lower yields for the berries of related *Juniperus* species: 0.7% for *J. communis* L. ssp. *nana* at 50 °C and 9 MPa [20], and 0.9% for *J. communis* extracted at 45 °C and 11.8 MPa [21]. The highest extraction yield

among junipers was obtained from the ripe fruits of *J. oblonga*, at 30 MPa and 311 K (37.85 °C), with an extraction yield of 7% [22]. These comparisons indicate that the JoB-SFE parameters employed in the present work co-extracted both volatile and non-volatile constituents and yielded an efficient extract from an economical point of view compared to the conventional methods.

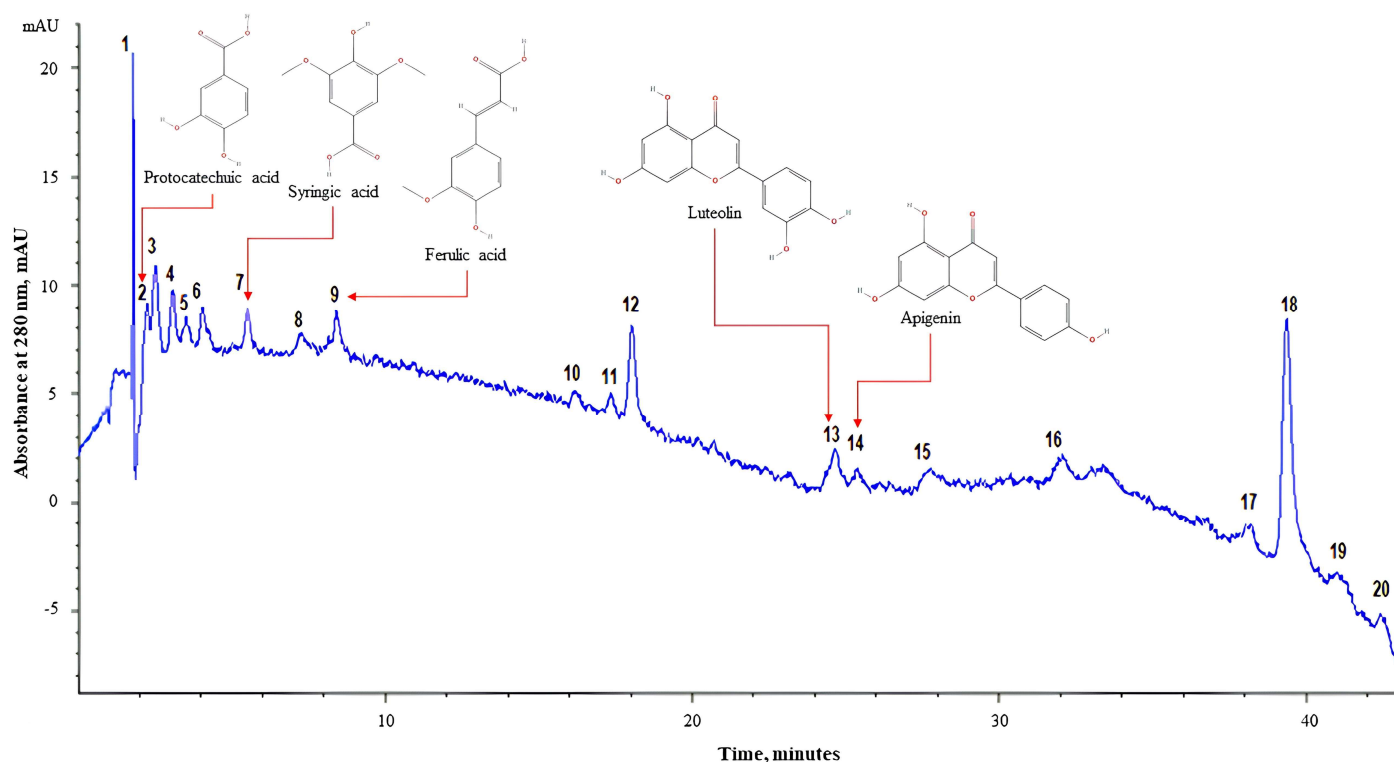
**Table 1.** Extraction yield, and global and individual phenolic characterizations.

| Assessment                                     | Result                   |
|------------------------------------------------|--------------------------|
| Extraction yield (%)                           | 4.35                     |
| Global phenolic characterization               |                          |
| TPC (mg GAE/g DW)                              | 16.42 ± 0.86             |
| TFC (mg QE/g DW)                               | 0.61 ± 0.02              |
| Individual phenolic characterization (µg/g DW) |                          |
| Ferulic acid                                   | 0.32 ± 0.01 <sup>D</sup> |
| Protocatechuic acid                            | 0.85 ± 0.03 <sup>A</sup> |
| Syringic acid                                  | 0.37 ± 0.02 <sup>D</sup> |
| Apigenin                                       | 0.47 ± 0.02 <sup>C</sup> |
| Luteolin                                       | 0.58 ± 0.04 <sup>B</sup> |

Note: the uppercase letters are used for statistical comparison between the RP-HPLC identified compounds, which means that those that do not share a letter are significantly different ( $p < 0.05$ ), according to Tukey's post hoc tests.

The global phytochemical characterization (Table 1) of the JoB-SFE extract revealed a total phenolic content (TPC) of 16.42 ± 0.86 mg GAE/g DW and a total flavonoid content (TFC) of 0.61 ± 0.02 mg QE/g DW. In our previous work [11], ultrasound-assisted extraction of the same *J. oxycedrus* raw material using a hydroethanolic solvent acidified with acetic acid showed lower values, with a TPC of 2.12 ± 0.05 mg GAE/g DW, and a TFC of 0.17 ± 0.09 mg QE/g DW. The markedly higher total phenolic and flavonoid contents obtained from the JoB-SFE extract compared to the ultrasound-assisted hydroethanolic extract suggest that the SFE provided more efficient solubilization of total lipophilic phenolic and flavonoid compounds.

In the phenolic profile chromatograph of the extract (Figure 1), the RP-HPLC sensor detector recorded 20 peaks. Among these, five phenolic compounds were successfully identified and quantified using the RP-HPLC system database at our research center. The remaining peaks failed to significantly match with available calibration curves with standard compounds available in our RP-HPLC system database and, as a result, could not be identified. Among the identified compounds (Table 1), protocatechuic acid was the predominant phenolic compound at a content of 0.85 ± 0.03 µg/g DW, followed by luteolin and apigenin (0.58 ± 0.04 and 0.47 ± 0.02 µg/g DW, respectively), whereas ferulic and syringic acids were present in lower concentrations.



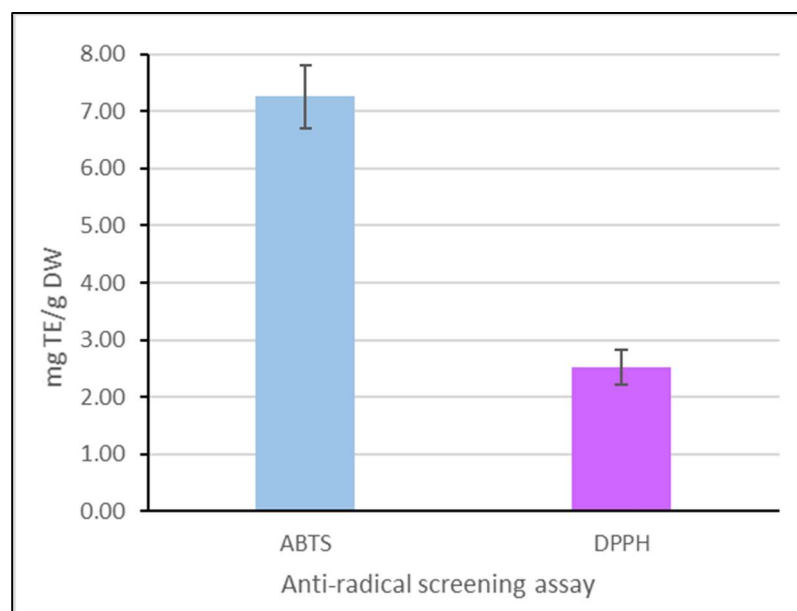
**Figure 1.** Phenolic profile chromatograph of juniper extract at 280 nm. Note: The 2D structures of the identified compounds were obtained from PubChem.

## 2.2. Antioxidant Activity and Correlation with Phenolic Content

The bar chart shown in Figure 2 illustrates the antioxidant activity of the JoB-SFE extract. The extract exhibited higher antioxidant activity in the ABTS assay ( $7.26 \pm 0.55$  mg TE/g DW) than in the DPPH assay ( $2.52 \pm 0.30$  mg TE/g DW). This difference reflects the distinct radical-scavenging mechanisms and solvent compatibilities of the two assays. The ABTS<sup>+</sup> radical cation is soluble in both aqueous and organic media and responds to a wide spectrum of antioxidants, including both hydrophilic and lipophilic compounds, while the DPPH radical is soluble mainly in organic solvents and reacts preferentially with lipophilic hydrogen donors. The ABTS assay involves both electron transfer and hydrogen atom transfer mechanisms, thus capturing the total antioxidant capacity of compounds across a broader polarity range [23,24]. Similar trends, where ABTS activity exceeds DPPH activity, have been reported previously. Mrid et al. [25] reported higher ABTS than DPPH activity responses in hydromethanolic and aqueous extracts of unripe JoB. In our previous work [11], the acidified hydroethanolic extract of JoB exhibited a similar pattern.

The antioxidant capacity of the extract was further evaluated through statistical analyses integrating the Pearson correlation to elucidate the relationships among the TPC, TFC, and antioxidant assays (ABTS and DPPH). The Pearson correlation analysis revealed that ABTS activity was strongly and positively correlated with TPC ( $r = 0.996$ ,  $p = 0.058$ ) and negatively correlated with TFC ( $r = -0.991$ ,  $p = 0.086$ ). Based on the investigated relationships between different classes of phenolic compounds and the antioxidant response from JoB-SFE, phenolic compounds appeared to play a dominant role in scavenging ABTS radicals, while flavonoids appeared to contribute inversely to this activity. In contrast, DPPH activity showed a strong positive correlation with TFC ( $r = 0.999$ ,  $p = 0.016$ ) and a negative correlation with TPC ( $r = -0.969$ ,  $p = 0.160$ ). This suggests that among the phenolic compounds examined, flavonoids are the primary contributors to the reduction in DPPH radicals. The significant positive correlation between DPPH and TFC ( $p < 0.05$ ) sustains

the quantitative relationship between flavonoid concentration and hydrogen-donating antioxidant capacity. However, it is important to note that the antioxidant activity in complex plant extracts results from multiple phytochemicals compounds that exert antioxidant potential [26].



**Figure 2.** Antioxidant activity of juniper extract.

### 2.3. Antibacterial Activity

#### 2.3.1. Minimum Inhibitory and Bactericidal Concentration

Table 2 presents the MIC and MBC of JoB-SFE against *Staphylococcus aureus* ATCC 25923, determined through microdilution assay. The extract exhibited an MIC of 1.56 mg/mL and an MBC of 6.25 mg/mL. To provide a reference framework, these values were compared with the clinical MIC breakpoints (Version 16.0, 2026) reported by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The lowest MIC of 0.001 mg/L was reported for fluoroquinolone antibiotics, ciprofloxacin and levofloxacin, while the highest of 16 mg/L was reported for amikacin [27]. Although these differences are several orders of magnitude, such variation is expected given that JoB-SFE is a crude plant extract, not a purified antibiotic compound. Nonetheless, the ability of JoB-SFE to inhibit *S. aureus* growth demonstrates the presence of bioactive constituents with antibacterial activity potential.

The MBC/MIC ratio is an important indicator of antibacterial action and classification. Bacteriostatic agents exhibit an MBC/MIC ratio greater than 4 and act as bacterial growth inhibitors. Meanwhile, bactericidal agents, with an MBC/MIC ratio of 4 or less, kill bacteria directly [28,29]. In our case, the MBC/MIC ratio is 4.00, which, according to the definitions, could be classified as bactericidal.

The bactericidal activity is clinically significant, as it directly kills bacterial cells, resulting in faster infection resolution and improved clinical outcomes. Rapid elimination of bacterial pathogens may also help reduce the development of antibiotic resistance and limit the spread of infections [29].

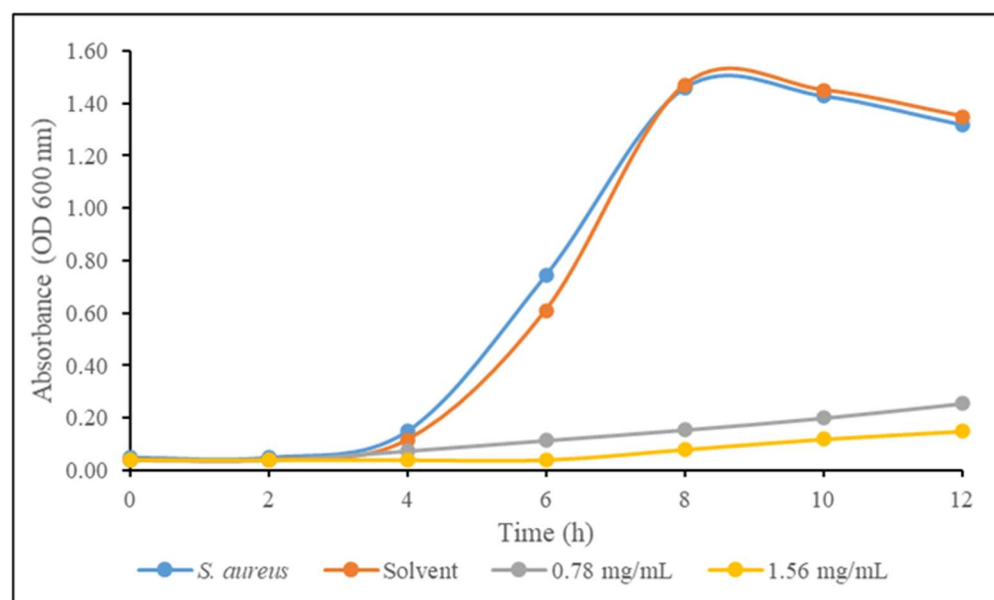
**Table 2.** Minimal Inhibitory and Bactericidal Concentration of juniper extract against *S. aureus*.

| Concentration of JoB-SFE (mg/mL) | 50.00 | 25.00 | 12.50 | 6.25 | 3.13 | 1.56 | 0.78 | 0.39 | 0.20 | 0.10 | 0.05 | 0.02 |
|----------------------------------|-------|-------|-------|------|------|------|------|------|------|------|------|------|
| Growth result                    | –     | –     | –     | MBC  |      | MIC  | +    | +    | +    | +    | +    | +    |

Note: “–” indicates absence of bacterial growth, while “+” indicates bacterial growth.

### 2.3.2. Growth Curve Kinetic

The growth kinetic of *S. aureus*, illustrated in Figure 3, reveal typical bacterial behavior in both control and solvent-treated cells, characterized by a short adaptation period followed by rapid exponential proliferation from 4 to 8 h and transitions into the stationary phase after 8 to 10 h. In contrast, exposure to JoB-SFE caused an inhibition of bacterial growth, manifested as a prolonged lag phase lasting 6 to 8 h and delayed entry into the exponential phase, highlighting the antibacterial properties of the extract at subinhibitory (0.78 mg/mL) and inhibitory (1.56 mg/mL) concentrations.

**Figure 3.** *Staphylococcus aureus* growth curb kinetic.

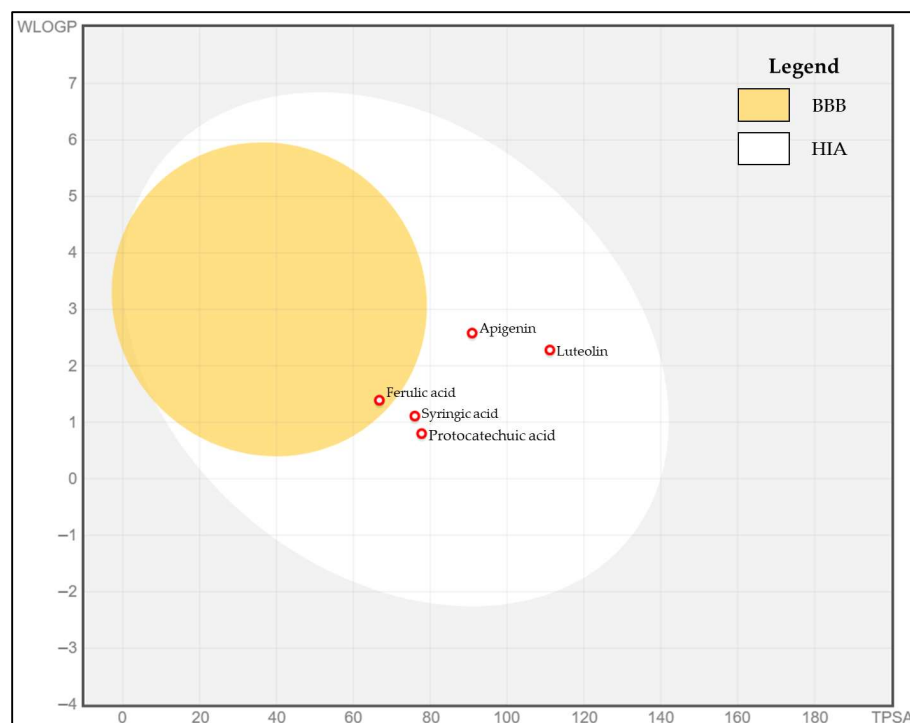
### 2.4. Bioinformatics Prediction of ADME and Toxicity of the Identified Phenolic Compounds

The bioinformatic tools, SwissADME and ProTox-3.0, were further used for assessing the absorption, distribution, metabolism, and excretion (ADME), as well as toxicity of the phenolic compounds identified through RP-HPLC analysis, such as to estimate the potential of using the JoB-SFE as a functional food ingredient, dietary supplement, or pharmacological agent. The results of the pharmacokinetic parameters predicted by the theoretical and computational models are presented in Figure 4 and Table A1 (in the Appendix A section).

The BOILED-Egg (Brain Or IntestinaL EstimateD) permeation predictive model, illustrated in Figure 4, provides a visual representation of the passive absorption and blood–brain barrier penetration of the investigated molecules identified in JoB-SFE. The white region of the model represents the physicochemical space for compounds with a high probability of human intestinal absorption (HIA), while the yellow region indicates the space for compounds likely to penetrate the blood–brain barrier (BBB) [30]. Among the five investigated phytochemicals, ferulic acid is situated in the BBB region, suggesting its potential to cross the blood–brain barrier and exert neuroprotective or cognitive-enhancing effects within the central nervous system. In contrast, the protocatechuic acid,

syringic acid, apigenin, and luteolin are located in the HIA area. This positioning suggests that, while they are likely to be highly absorbed in the gastrointestinal tract, they may not reach significant concentrations in the brain, indicating that their effects are more likely peripheral.

The SwissADME results (Table A1) indicate that the phenolic compounds prevailing in the JoB-SFE possess physicochemical characteristics with favorable pharmacokinetic behavior. All compounds have molecular weights ranging between 154.12 and 286.24 g/mol, complying with Lipinski's rule of five and indicating potential oral bioavailability. Their number of hydrogen bond donors (between 2 and 4) and acceptors (between 4 and 6), along with moderate lipophilicity (WLOGP between 0.80 and 2.58), support good membrane permeability and solubility balance. The topological polar surface area (TPSA) values ranging from 66.76 to 111.13 Å<sup>2</sup> fall within the compatible range of effective highly gastrointestinal (GI) absorption. According to the SwissADME predictions, none of the investigated compounds are substrates for P-glycoprotein (P-gp). This suggests that the compounds prevailing in the JoB-SFE are less likely to be effluxed out of cells, resulting in greater absorption and systemic bioavailability after oral intake. P-gp stands for the glycosylated membrane-bound protein found throughout the body (intestine, liver, kidney, pancreas, brain, and placenta) with particularly high expression in the epithelial cells of the colon and ileum. During the process of oral absorption, the properties of pharmacological agents (solubility and permeability), along with P-gp efflux across the intestinal apical membrane, determine the rate and quantity of active agents that diffuse across the basolateral membrane into general circulation. Therefore, evaluating P-gp efflux is a crucial step in the preliminary screening stage of pharmacological agent discovery and development [31]. The bioavailability scores (between 0.55 and 0.85) are moderate to high, with ferulic acid showing the most favorable pharmacokinetic profile.



**Figure 4.** The BOILED-Egg model prediction for the identified phenolic compounds. Note: “HIA” indicates human intestinal absorption, while “BBB” indicates the blood–brain barrier.

The ProTox-3.0 toxicity predictions (Table A1) suggest that all investigated compounds have a generally safe profile. The lowest predicted median lethal dose (LD<sub>50</sub>), which is the amount at which 50% of test subjects die from exposure to a compound, was estimated at 1700 mg/kg body weight for syringic acid, with an accuracy of 69.26%. Meanwhile, the highest LD<sub>50</sub> was estimated at 3919 mg/kg body weight for luteolin, with an accuracy of 70.97%. It is important to note that these LD<sub>50</sub> values are predicted for single pure compounds. Considering the quantified content of each compound in the JoB-SFE, as well as the possible quantities in dietary products or applied as pharmacological agents, the likelihood of oral toxicity is low.

None of the phytochemicals were predicted to be hepatotoxic, neurotoxic, or cardiotoxic, suggesting a low risk for side effects in these organs with probabilities ranging from 0.51 to 0.99. However, all compounds displayed potential nephrotoxic activity, with moderate probabilities between 0.60 and 0.66. This prediction may reflect the renal excretion pathway typically associated with phenolic metabolites rather than a true toxic effect [32]. Nonetheless, caution is advised in cases of concentrated doses or chronic exposure. Additionally, apigenin and luteolin exhibited potential respiratory toxicity, with probabilities ranging from 0.58 to 0.83.

Carcinogenicity predictions indicated a positive risk for protocatechuic acid and luteolin, with probabilities of 0.72 and 0.68, respectively. However, the literature supports their anticarcinogenic and cytoprotective roles [33–35], suggesting that these *in silico* alerts may reflect limitations of the model rather than the actual risk. All compounds were predicted to be non-cytotoxic; however, ferulic and syringic acids were predicted to exceed clinical toxicity with probabilities of 0.52 and 0.55, respectively. Additionally, ferulic acid and apigenin were predicted to be immunotoxic and exotoxic (probability of 0.91), respectively, while luteolin was predicted to present nutritional toxicity (probability of 0.63).

Analysis of the Tox21 receptor pathway predictions highlighted significant biological interactions for apigenin and luteolin. Both compounds appeared to be active toward the aryl hydrocarbon receptor (AhR), estrogen receptors (ER $\alpha$  and ER-LBD), and the peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ), as also from literature reports [35–37]. These pathways are essential for antioxidant defense, hormonal regulation, and metabolic balance, providing mechanistic support for their reported anti-inflammatory, anti-diabetic, and anticancer properties [38–40].

Furthermore, apigenin was predicted to interact with the mitochondrial membrane potential (MMP) and p53 tumor suppressor pathways, suggesting a potential for inducing apoptosis and chemopreventive activity.

Predictions regarding cytochrome P450 interactions indicate that all the investigated phenolic compounds are likely substrates or modulators of CYP2C9, with probabilities ranging from 0.54 to 0.99. Apigenin and luteolin potentially interact with CYP1A2 and CYP2C19, with probabilities ranging between 0.77 and 1.00. Additionally, apigenin may potentially interact with CYP3A4, showing a high probability of 0.99, suggesting metabolic involvement and potential for drug–nutrient interactions. In contrast, protocatechuic, ferulic, and syringic acids were potentially inactive with CYP isoforms, indicating faster metabolism and reduced interaction potential. While cytochrome P450 modulation could affect drug metabolism at pharmacological doses, such effects are less likely to occur at dietary concentrations. Furthermore, flavonoid-mediated regulation of CYP enzymes may offer hepatoprotective and detoxifying benefits, thereby enhancing the functional value of the extract [41,42]. However, Křížková et al. [42], noted that while selected flavonoids can modulate the activity of cytochromes P450 in rat liver and small intestine, the intake of CYP1A inducers might increase the risk of cancer development in humans.

Therefore, the consumption of dietary supplements containing flavonoids without limitations should be approached with caution.

### 3. Discussion

A bibliometric analysis examining publications from 1995 to 2024 reveals a continued research interest in using SFE for extracting phytochemical compounds from aromatic and medicinal plants. This analysis highlights the broader selectivity of SFE compared to traditional steam or hydrodistillation techniques [19].

Related to global characterization, comparative data from the literature show substantial variations depending on the extraction method, solvent polarity, and plant maturity. Mrid et al. [25] investigated unripe JoB extracted using maceration with hydro-methanolic and aqueous solvents, reporting TPC values of  $28.11 \pm 3.11$  and  $131.48 \pm 4.58$  mg GAE/g DW, and TFC values of  $3.20 \pm 0.79$  and  $8.28 \pm 0.74$  mg QE/g DW, respectively. Similarly, Živić et al. [43] obtained TPC values ranging from  $15.68 \pm 0.14$  to  $58.73 \pm 0.14$  mg GAE/g DW in Soxhlet extracts of JoB using ethanol, ethyl acetate, and chloroform as extraction solvents. These variations in content are attributable to differences in solvent compositions, extraction techniques, and geographical environmental factors.

In terms of individual phytochemical characterization, a comparison with previously published studies highlights significant differences in phenolic composition, reflecting the influence of extraction technique and solvent polarity. Zlatanović et al. [44] identified protocatechuic acid (5.36 mg/g DE), rutin (0.73 mg/g DE), apigenin (0.31 mg/g DE), cupressoflavone (3.25 mg/g DE), amentoflavone (3.70 mg/g DE), and a biflavone derivative (1.40 mg/g DE) in JoB extract obtained by ultrasound-assisted maceration using methanol as the solvent, whereas syringic acid and luteolin derivatives were not detected. In contrast, Kachmar et al. [45] reported the presence of protocatechuic acid glucoside isomer and ferulic acid glucuronide isomer in the JoB infusion. However, their concentrations were below the quantification limit of HPLC-PDA/ESI-MS. Similarly, Mrid et al. [25] detected caffeic acid, salicylic acid, *p*-hydroxybenzoic acid, hesperidin, naringenin, rutin, and thymoquinone in unripe JoB extract analyzed by HPLC-DAD, but syringic acid was also absent in the extract.

Taken together, these results suggest that the SFE conditions used in the present study favored the selective recovery of low- and medium-polar phenolic acids and flavonoids (protocatechuic acid, luteolin) while excluding high-polar glycosides that are more efficiently extracted by aqueous or hydroalcoholic solvents.

Antioxidant agents are important mediators for cellular redox processes, which maintain cellular homeostasis. Free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), can emerge from normal physiological processes or diseases, serving both beneficial and harmful roles, such as triggering cancer cell apoptosis or promoting cancer cells. The body regulates free radicals through various signaling pathways to prevent cellular damage. In this context, a balance between enzymatic and non-enzymatic antioxidants is vital for maintaining redox homeostasis. Increased ROS production or reduced antioxidant efficiency leads to oxidative stress, which is linked to all stages of cancer development. Consequently, antioxidants are being studied as potential cancer prevention and treatment options. Non-enzymatic antioxidants, such as flavonoids, carotenoids, and vitamins A, C, and E, as well as uric acid and glutathione, help protect cells from excess free radicals. *J. oxycedrus* berries are particularly rich in free radical-scavenging compounds and exhibit high antioxidant activity. Incorporating these phenolic compounds into the diet may enhance cellular defenses and also offer therapeutic benefits worth exploring [25,46].

The MIC and MBC results obtained from our JoB-SFE demonstrated stronger antibacterial activity than some of the works previously reported for *J. oxycedrus* extracts. For

instance, Ertürk [13] reported a MIC value of 5 mg/mL against *S. aureus* ATCC 25923 using hydroethanolic extract, while in our prior study [11], the acidified hydroethanolic extract showed a MIC of 6.25 mg/mL and a MBC of 12.50 mg/mL. The lower MIC can be attributed to the efficiency of supercritical CO<sub>2</sub> to extract selective bioactive compounds of volatile terpenoids and non-volatile lipophilic compounds with antimicrobial properties.

The antimicrobial activity of bioactive compounds can occur through various mechanisms, including (a) disruption or structural modification of the cell membrane; (b) alteration of gene expression; (c) impact on DNA, RNA, and certain proteins essential for cell function; (d) chelation of metal ions, which affects the activity of ATP synthase; and (e) influence on cellular metabolic pathways, leading to reduced cell efficiency and ultimately causing bacterial death [47].

Similar findings have been reported in the literature regarding antibacterial activity of plant extracts against *S. aureus*. Zhou et al. [48] studied the effect of *Chimonanthus salicifolius* S. Y. Hu hydroethanolic extract on *S. aureus* ATCC 25923. Their extract demonstrated MIC and MBC values of 1.25 mg/mL and 6.25 mg/mL, respectively, which are similar to those reported in our study for JoB-SFE. The authors reported that the lag phase of *S. aureus* treated with the MIC concentration was extended by 8 h. Additionally, the study suggested that the extract caused disruption of the cell wall membrane, leakage of intracellular components, DNA damage, and alterations in gene expression.

The presence of phenolic acids (ferulic and syringic acid) and flavonoid aglycones (luteolin and apigenin) detected in the JoB-SFE may further contribute to its bactericidal effect through membrane destabilization, enzyme inhibition, and interference with bacterial signaling pathways [47,49]. Spengler et al. [12] studied the effects of EO from the aerial parts of *J. oxycedrus* ssp. *macrocarpa* against *S. aureus* ATCC 25923, MRSA ATCC 44300, and other microorganisms. The EO of *J. oxycedrus* had limited activity against biofilm production, but exhibited MIC-modulating effects when combined with certain antibiotics against *S. aureus* and the MRSA strain. The most pronounced synergistic effect was observed with ciprofloxacin, where MIC values were reduced 4 to 8 times when the EO was applied at a fixed concentration of 0.25%. Additionally, the EO displayed strong efflux pump inhibitory properties in the case of *S. aureus* and *Escherichia coli*.

In modern medicine, drugs are primarily developed and synthesized as single bioactive compounds designed to target specific mechanisms. In contrast, traditional medicine utilizes MAP, which provides a wide variety of bioactive compounds. This diversity offers the potential for synergism and multiple targeting mechanisms [8]. Synergism occurs when two or more agents combine to produce a greater effect than they would individually. This approach is particularly useful for treating infections linked to multidrug resistance or when a single-agent treatment may fail. Combining plant extracts/infusion between them or with pharmacological drugs can enhance their effectiveness by achieving synergism and acting on multiple targets, reducing the dosages needed, and minimizing side effects [12,29]. In this context, the JoB-SFE may serve as a promising source of a synergistic agent to enhance antibiotic action and mitigate resistance development.

The combined results from SwissADME and ProTox-3.0 predictions support the safety and functionality of the JoB-SFE in terms of the investigated phytochemical compounds. The phenolic compounds exhibit high gastrointestinal absorption, low systemic toxicity, and favorable receptor activity profiles. The safety predictions on hepatic, neuronal, and cardiac systems, along with antioxidant and anti-inflammatory properties, reinforce their suitability for nutraceutical and therapeutic applications. However, the mild nephrotoxicity predictions suggest that caution should be exercised regarding dosage and long-term exposure, highlighting the need for further in vitro and in vivo validation. Additionally, the metabolic involvement of apigenin and luteolin suggests that drug interaction studies should be further elucidated.

It is important to note that the predictions obtained by SwissADME and ProTox-3.0 refer to individual compounds, regardless of their concentration in the extracts. Moreover, these predictions do not take into account the matrix effects or compound interactions, whether synergistic or antagonistic, and may not fully reflect the pharmacokinetic behavior of the plant extract complex mixture. Therefore, the conclusions drawn from these *in silico* predictions require validation through *in vitro* and *in vivo* studies to confirm the safety, stability, and functional efficacy of the extract in actual food systems or synergistic pharmacological agents.

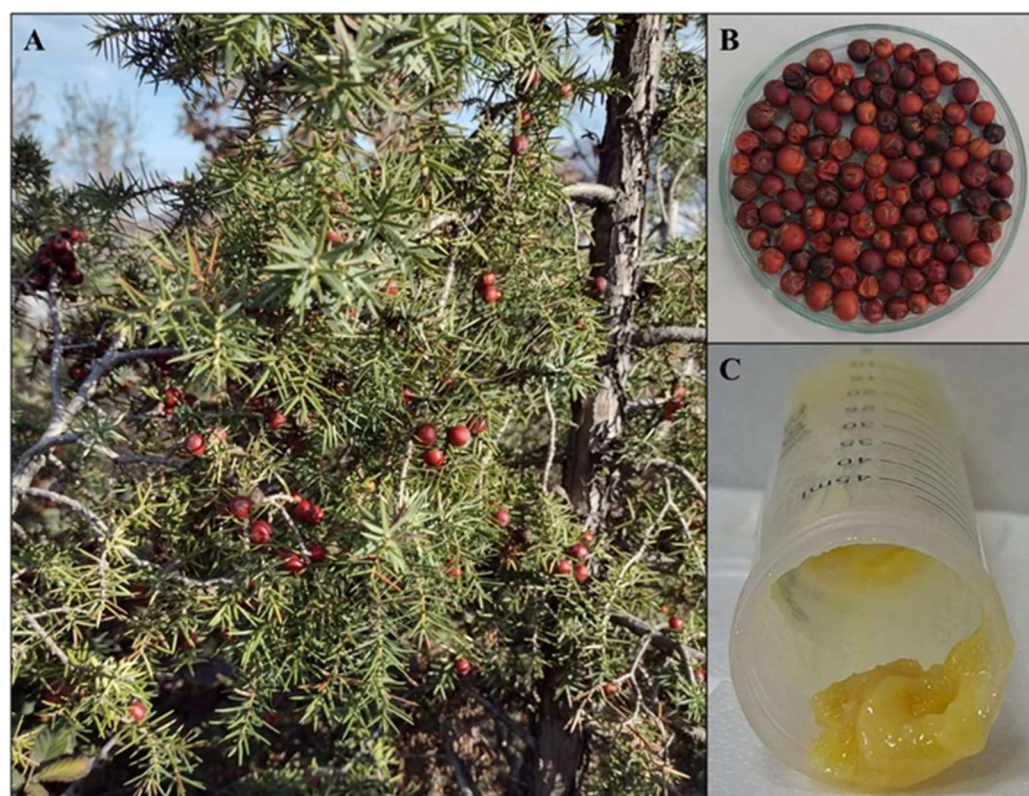
## 4. Materials and Methods

### 4.1. Reagents

The reagents used for this study were as follows: aluminum chloride, sodium carbonate, diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), standards for HPLC, and dimethyl sulfoxide anhydrous (99.9%) were purchased from Sigma-Aldrich (Sigma-Aldrich; Damstadt, Germany). Tween-40 (Sigma-Aldrich; St. Louis, MO, USA), methanol, formic acid, acetonitrile, and ethyl acetate of HPLC grade were purchased from Honeywell (Honeywell; Seelze, Germany). From Bucharest, Romania, the following were purchased: methanol, ethanol of analytical grade (S.C. Chimreactiv, S.R.L.; Bucharest, Romania), Folin–Ciocâlteu (Remed Prodimpex S.R.L.; Bucharest, Romania), and CO<sub>2</sub> (99.997% *w/w*) used for supercritical fluid extraction (Messer S.A.; Bucharest, Romania). The media used for microbiological assays include Brain Heart Infusion Broth (Millipore, Sigma-Aldrich, Damstadt, Germany), Mueller–Hinton Broth (Scharlab S.L., Gato Perez, Spain), and Agar (Scharlau Chemie S.A.; Barcelona, Spain). Water of purity 0.058 µS/cm was obtained from the SMART N-II Heal Force Purification System (Shanghai, China).

### 4.2. Plant Material

The wild *Juniperus oxycedrus* berries, shown in Figure 5A and identified by Prof. Dr. Lulëzim Shuka (Department of Biology; Faculty of Natural Science; University of Tirana; Albania), were collected in Albania in December 2022 in Has, Kukës (42°16'44.0" N 20°22'15.1" E), as initially reported [11]. The berries were dried at room temperature. The dried berries (Figure 5B) sample had a moisture content of 14.73 ± 0.00%, water activity value of  $a_w = 0.52 \pm 0.00$ , and were kept in airtight paper containers that were stored in the dark at ambient temperature until the extraction.



**Figure 5.** Wild *Juniperus oxycedrus* tree (A), the dried berries (B), and the concentrated JoB-SFE (C).

#### 4.3. Supercritical Fluid Extraction

Prior to extraction, the dried berries were ground using an electric grinder (Heinner HCG-150SS; Bucharest, Romania). The supercritical extraction of the bioactive compounds from the berries was performed in pilot-plant equipment manufactured by Natex (Prozesstechnologie GesmbH, Fabrication No. 10-023/2011; Ternitz, Austria) utilizing pure CO<sub>2</sub> as a carrier. The pilot plant features a 2.0 L stainless steel extractor vessel along with two separators, S40 and S45, each with a volume of 1.5 L. Both separators have independent controls for temperature and pressure. The extraction conditions involved a temperature of 45 °C, a pressure of 30 MPa, and a dynamic extraction period of 1.5 h, adapted from the literature studying similar raw material [21,50]. The extraction batch consisted of 300 g of ground dried sample mixed with Raschig rings at a 1:1 *w/w* ratio before being loaded into the extractor vessel. The CO<sub>2</sub> flow rate was 22.8 kg/h, and it was monitored using a Sitrans F C Mass 2100 flow meter (Siemens A/S Flow Instruments; Nordborg, Denmark). Based on previous experience with similar raw material, it was decided that the extracts from the two separators would be combined after being collected from the separators using ethanol. The extract was centrifuged at 7000 rpm for 10 min at 4 °C (Universal 320R; Hettich, Germany), and the supernatant was concentrated using a rotary vacuum concentrator with an integrated vacuum pump and cooling trap (RVC 2-18 CDplus; Martin Christ, Germany) until constant weight. Following solvent evaporation, the extraction yield was determined gravimetrically as a percentage (% *w/w*) of the concentrated extract relative to the initial raw material. The concentrated extract (Figure 5C) was stored at 4 °C in a dark container until further analysis.

#### 4.4. Global Phytochemical Characterization and Antioxidant Activity

The global phytochemical characterization and antioxidant activity of the SFE were assessed using spectrophotometric methods (Biochrom; Libra 22 UV–Vis Spectrophotometer; Holliston, MA, USA), following the procedures described previously [11].

Concentrated extracts were redissolved in 70% ethanol (*v/v*) prior to analysis. Total phenolic content (TPC) is expressed as milligrams of gallic acid equivalents per gram of dry weight (DW) of concentrated extract (mg GAE/g DW). Total flavonoid content (TFC) is expressed in milligrams of quercetin/g DW (mg QE/g DW). The antioxidant activity was determined using the DPPH and ABTS radical-scavenging assays, with results expressed in milligrams of Trolox equivalent/g DW (mg TE/g DW). Results were calculated from calibration curves and presented as mean values of triplicate measurements  $\pm$  standard deviation (SD).

#### 4.5. Chromatographic Characterization of Phenolic Compounds

The individual phenolic compounds in the extract were analyzed using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). The characterization was conducted on an Agilent 1200 HPLC system equipped with an autosampler, degasser, quaternary pump, multi-wavelength detector, and column thermostat (Agilent Technologies; Santa Clara, CA, USA) following a previously described protocol [16,51]. Identified compounds were quantified using calibration curves of standard references, with results expressed as  $\mu\text{g/g}$  DW and reported as mean values of duplicate measurements  $\pm$  SD.

#### 4.6. Determination of Minimal Inhibitory and Bactericidal Concentration

To evaluate the antibacterial activity of the concentrated extract against *Staphylococcus aureus* ATCC 25923, a stock solution was prepared by dissolving the concentrated extract (100 mg/mL) in a mixture of solvents prepared in ultrapure water, including acetone (5%), DMSO (7%), and Tween-40 (15%).

The working bacterial culture was prepared by resuspending one colony of *S. aureus* in 10 mL of Brain Heart Infusion (BHI) broth and incubating it overnight at 37 °C. The inoculum size of the overnight culture was determined by measuring the absorbance at 600 nm using a Biowave CO8000 cell density meter (Biochrom Ltd., Cambridge, UK). This measurement was correlated with the number of colonies grown by inoculating 1 mL of a decimal dilution into BHI agar media. The overnight culture was estimated to have a concentration of  $10^7$  CFU/mL.

The MIC of JoB-SFE was determined using a microdilution assay in a 96-well microplate. A serial two-fold dilution was performed by mixing 100  $\mu\text{L}$  of the extract with 100  $\mu\text{L}$  of Mueller–Hinton (MH) broth media, resulting in concentrations ranging from 50.00 mg/mL to 0.02 mg/mL. To each well, 100  $\mu\text{L}$  of diluted overnight *S. aureus* culture ( $5 \times 10^5$  CFU/mL, as advised in ISO 20776-1:2019 [52]) and 10  $\mu\text{L}$  of resazurin solution (2 mg/mL) were added. Control conditions included the bacteria culture treated with 10  $\mu\text{L}$  of ampicillin (10 mg/mL) and 10  $\mu\text{L}$  of erythromycin (10 mg/mL) as a negative control, along with a positive control that included the culture treated with the same solubilization solvent mixture, as in the case of the working samples, to validate the assay. The microplate was incubated for 18 h at 37 °C. To assess bacterial growth inhibition without interference from the color of So-SFE affecting the visual changes of resazurin due to bacterial metabolic activity, 10  $\mu\text{L}$  from each well was inoculated onto the surface of an MH agar plate and incubated for an additional 24 h at 37 °C. The MIC was defined as the lowest concentration of the extract at which bacterial growth was inhibited [16]. Meanwhile, to determine the MBC, 100  $\mu\text{L}$  from each well of the microplate was further inoculated in 900  $\mu\text{L}$  of fresh BHI and incubated for an additional 24 h at 37 °C. Following this incubation, 10  $\mu\text{L}$  was transferred to the surface of a BHI agar plate and incubated for 24 h at 37 °C [53]. The MBC was defined as the lowest concentration of the extract at which no bacterial growth was observed.

#### 4.7. Growth Curve Kinetics

The influence of JoB-SFE on the growth kinetic of *S. aureus* was investigated by following previously reported methods [16,54,55]. The overnight culture of *S. aureus*, prepared as described in Section 4.6, was inoculated into BHI broth to achieve an initial optical density (OD<sub>600</sub>) of  $0.04 \pm 0.01$ . The extract was then added at inhibitory and subinhibitory concentrations. The solvent used to solubilize the extract was added to the media under the same conditions and served as a control to validate the assay. The cultures were incubated at 37 °C, and bacterial growth was monitored spectrophotometrically using a cell density meter (Biowave CO8000; Biochrom Ltd., Cambridge, UK) by measuring absorbance at 600 nm every two hours for a total of 12 h.

#### 4.8. In Silico ADME and Toxicity Estimation

The predictions of the ADME for the identified phenolic compounds in the JoB-SFE were performed on the SwissADME online server (<https://www.swissadme.ch/> 28 January 2026). Meanwhile, toxicity predictions were conducted using the ProTox-3.0 online server (<https://tox.charite.de/protox3/> 28 January 2026). The SMILES codes of the phenolic compounds for the in silico assessment were obtained from the PubChem database (last accessed on 28 January 2026) according to Güven and Hancı [56].

#### 4.9. Statistical Analyses

Statistical analyses were conducted using Minitab software (Version 19.1 for Windows). The data were tested for normality with the Ryan–Joiner test. One-Way ANOVA was performed to compare concentration differences among the identified RP-HPLC compounds, with Tukey’s post hoc test ( $p > 0.05$ ) at a 95% confidence level. The relationships between phenolic content and antioxidant assays were investigated by performing a Pearson correlation.

### 5. Conclusions

The results obtained from *Juniperus oxycedrus* berries extracted using supercritical fluid extraction (JoB-SFE) indicate that the extract possesses antioxidant and antibacterial potential. Statistical analysis suggests that, from the investigated compounds, the extract’s antioxidant potential arises from distinct classes of phytochemicals, with phenolic compounds primarily influencing the ABTS scavenging assay and flavonoids on the DPPH scavenging assay. The in vitro antibacterial activity against *Staphylococcus aureus* supports the idea that the phytochemical compounds extracted from the JoB-SFE, depending on the concentration, delay bacterial cell replication, inhibit growth, and lead to bacterial death. These findings suggest potential applications of the extract as an alternative or complementary agent for controlling and preventive strategies against *S. aureus*. The bioinformatics investigations focused on the phytochemical compounds identified in *J. oxycedrus* extract indicated the potential as a candidate for functional foods, dietary supplements, and pharmacological applications.

However, further experimental evaluations are necessary to fully characterize the extract, elucidate the synergistic effects, investigate the mechanisms responsible for the antimicrobial properties, and determine safety margins and bioactivities.

**Author Contributions:** I.M.: writing—original draft, review and editing, conceptualization, methodology, investigation, formal analysis, validation, visualization, software, and data curation. L.G.-G.: writing—review and editing, methodology, investigation, formal analysis, and validation. L.M.: writing—review and editing, methodology, investigation, validation, software, and resources. I.A.: writing—review and editing, validation, visualization, software, resources, and funding acquisition. M.T.: methodology, investigation, formal analysis, validation, and software. G.R.: writing—

review and editing, resources, and funding acquisition. N.S.: writing—review and editing, validation, supervision, project administration, resources, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by a grant from the Ministry of Research, Innovation and Digitization, CNCS/CCCDI-UEFISCDI, project number ERANET-M-3-SMARTGEL, within PNCDI IV.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

**Acknowledgments:** The Integrated Center for Research, Expertise, and Technological Transfer in the Food Industry is acknowledged for providing technical support.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Appendix A

**Table A1.** SwissADME and ProTox-3.0 predictions for the identified phenolic compounds in JoB-SFE.

|                            |                          | Protocatechuic Acid                          | Ferulic Acid                                   | Syringic Acid                                 | Apigenin                                       | Luteolin                                       |
|----------------------------|--------------------------|----------------------------------------------|------------------------------------------------|-----------------------------------------------|------------------------------------------------|------------------------------------------------|
|                            | Formula                  | C <sub>7</sub> H <sub>6</sub> O <sub>4</sub> | C <sub>10</sub> H <sub>10</sub> O <sub>4</sub> | C <sub>9</sub> H <sub>10</sub> O <sub>5</sub> | C <sub>15</sub> H <sub>10</sub> O <sub>5</sub> | C <sub>15</sub> H <sub>10</sub> O <sub>6</sub> |
| Physicochemical Properties | Molecular weight (g/mol) | 154.12                                       | 194.18                                         | 198.17                                        | 270.24                                         | 286.24                                         |
|                            | Heavy atoms              | 11                                           | 14                                             | 14                                            | 20                                             | 21                                             |
|                            | Aromatic heavy atoms     | 6                                            | 6                                              | 6                                             | 16                                             | 16                                             |
|                            | Nr. Rotatable bonds      | 1                                            | 3                                              | 3                                             | 1                                              | 1                                              |
|                            | Nr. H-bond acceptors     | 4                                            | 4                                              | 5                                             | 5                                              | 6                                              |
|                            | Nr. H-bond donors        | 3                                            | 2                                              | 2                                             | 3                                              | 4                                              |
|                            | TPSA (Å <sup>2</sup> )   | 77.76                                        | 66.76                                          | 75.99                                         | 90.90                                          | 111.13                                         |
| BOILED-Egg Model Outputs   | WLOGP (Log Po/w)         | 0.80                                         | 1.39                                           | 1.11                                          | 2.58                                           | 2.28                                           |
|                            | GI absorption            | High                                         | High                                           | High                                          | High                                           | High                                           |
|                            | BBB permeant             | –                                            | +                                              | –                                             | –                                              | –                                              |
|                            | P-gp substrate           | –                                            | –                                              | –                                             | –                                              | –                                              |
|                            | Bioavailability Score    | 0.56                                         | 0.85                                           | 0.56                                          | 0.55                                           | 0.55                                           |
|                            | LD <sub>50</sub> (mg/kg) | 2000                                         | 1772                                           | 1700                                          | 2500                                           | 3919                                           |
| Oral toxicity              | Toxicity Class           | IV                                           | IV                                             | IV                                            | V                                              | V                                              |
|                            | Accuracy (%)             | 70.97                                        | 70.97                                          | 69.26                                         | 70.97                                          | 70.97                                          |
| Organ toxicity             | Hepatotoxicity           | Pred.                                        | –                                              | –                                             | –                                              | –                                              |
|                            |                          | Prob.                                        | 0.59                                           | 0.51                                          | 0.58                                           | 0.69                                           |
|                            | Neurotoxicity            | Pred.                                        | –                                              | –                                             | –                                              | –                                              |
|                            |                          | Prob.                                        | 0.86                                           | 0.74                                          | 0.76                                           | 0.89                                           |
|                            | Nephrotoxicity           | Pred.                                        | +                                              | +                                             | +                                              | +                                              |
|                            |                          | Prob.                                        | 0.61                                           | 0.62                                          | 0.60                                           | 0.62                                           |
|                            | Respiratory toxicity     | Pred.                                        | –                                              | –                                             | +                                              | +                                              |
|                            |                          | Prob.                                        | 0.58                                           | 0.77                                          | 0.75                                           | 0.83                                           |
|                            | Cardiotoxicity           | Pred.                                        | –                                              | –                                             | –                                              | –                                              |
|                            |                          | Prob.                                        | 0.90                                           | 0.85                                          | 0.91                                           | 0.99                                           |
|                            | Carcinogenicity          | Pred.                                        | +                                              | –                                             | –                                              | +                                              |
|                            |                          | Prob.                                        | 0.72                                           | 0.61                                          | 0.62                                           | 0.68                                           |
| Toxicity endpoints         | Immunotoxicity           | Pred.                                        | –                                              | –                                             | –                                              | –                                              |
|                            |                          | Prob.                                        | 0.99                                           | 0.91                                          | 0.97                                           | 0.97                                           |

|                                           |                      |       |      |      |      |      |      |
|-------------------------------------------|----------------------|-------|------|------|------|------|------|
|                                           | Mutagenicity         | Pred. | –    | –    | –    | –    | +    |
|                                           |                      | Prob. | 0.97 | 0.96 | 0.93 | 0.57 | 0.51 |
|                                           | Cytotoxicity         | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.90 | 0.88 | 0.97 | 0.87 | 0.99 |
|                                           | Ecotoxicity          | Pred. | –    | –    | –    | +    | –    |
|                                           |                      | Prob. | 0.82 | 0.87 | 0.83 | 0.51 | 0.53 |
|                                           | Clinical toxicity    | Pred. | –    | +    | +    | –    | –    |
|                                           |                      | Prob. | 0.51 | 0.52 | 0.55 | 0.54 | 0.53 |
|                                           | Nutritional toxicity | Pred. | –    | –    | –    | –    | +    |
|                                           |                      | Prob. | 0.80 | 0.82 | 0.86 | 0.55 | 0.63 |
| Tox21-Nuclear receptor signaling pathways | AhR                  | Pred. | –    | –    | –    | +    | +    |
|                                           |                      | Prob. | 0.96 | 0.94 | 0.88 | 1.00 | 0.91 |
|                                           | AR                   | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.84 | 0.83 | 0.99 | 0.99 | 0.99 |
|                                           | AR-LBD               | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 1.00 | 0.99 | 1.00 | 1.00 | 0.97 |
|                                           | Aromatase            | Pred. | –    | –    | –    | +    | –    |
|                                           |                      | Prob. | 0.99 | 0.99 | 1.00 | 0.61 | 0.91 |
|                                           | ER $\alpha$          | Pred. | –    | –    | –    | +    | +    |
|                                           |                      | Prob. | 0.99 | 0.96 | 0.83 | 1.00 | 0.87 |
|                                           | ER-LBD               | Pred. | –    | –    | –    | +    | +    |
|                                           |                      | Prob. | 0.95 | 0.96 | 0.89 | 1.00 | 0.95 |
|                                           | PPAR- $\gamma$       | Pred. | –    | –    | –    | +    | –    |
|                                           |                      | Prob. | 1.00 | 0.94 | 0.96 | 1.00 | 0.98 |
| Tox21-Stress response pathways            | Nrf2/ARE             | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.98 | 0.90 | 0.92 | 0.99 | 0.99 |
|                                           | HSE                  | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.98 | 0.90 | 0.92 | 0.99 | 0.99 |
|                                           | MMP                  | Pred. | –    | –    | –    | +    | +    |
|                                           |                      | Prob. | 0.99 | 0.92 | 0.78 | 1.00 | 1.00 |
|                                           | p53                  | Pred. | –    | –    | –    | +    | –    |
|                                           |                      | Prob. | 0.99 | 0.93 | 0.98 | 1.00 | 0.97 |
|                                           | ATAD5                | Pred. | –    | –    | –    | +    | –    |
|                                           |                      | Prob. | 1.00 | 0.93 | 0.95 | 0.96 | 0.99 |
| Molecular Initiating Events               | THR $\alpha$         | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 |
|                                           | THR $\beta$          | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
|                                           | TTR                  | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 |
|                                           | RYR                  | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 |
|                                           | GABAR                | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 |
|                                           | NMDAR                | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.92 | 0.92 | 0.92 | 0.92 | 0.92 |
|                                           | AMPAR                | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 |
|                                           | KAR                  | Pred. | –    | –    | –    | –    | –    |
|                                           |                      | Prob. | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
|                                           | AChE                 | Pred. | –    | –    | –    | –    | –    |

|                                         |         |       |      |      |      |      |      |
|-----------------------------------------|---------|-------|------|------|------|------|------|
| Metabolism<br>(Cytochrome P450 enzymes) | CAR     | Prob. | 0.59 | 0.51 | 0.58 | 0.68 | 0.69 |
|                                         |         | Pred. | –    | –    | –    | –    | –    |
|                                         | PXR     | Prob. | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 |
|                                         |         | Pred. | –    | –    | –    | –    | –    |
|                                         | NADHOX  | Prob. | 0.92 | 0.92 | 0.92 | 0.92 | 0.92 |
|                                         |         | Pred. | –    | –    | –    | –    | –    |
|                                         | VGSC    | Prob. | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 |
|                                         |         | Pred. | –    | –    | –    | –    | –    |
|                                         | NIS     | Prob. | 0.95 | 0.95 | 0.95 | 0.95 | 0.95 |
|                                         |         | Pred. | –    | –    | –    | –    | –    |
|                                         | CYP1A2  | Prob. | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 |
|                                         |         | Pred. | –    | –    | –    | +    | +    |
|                                         | CYP2C19 | Prob. | 0.97 | 0.95 | 0.89 | 1.00 | 1.00 |
|                                         |         | Pred. | –    | –    | –    | +    | +    |
|                                         | CYP2C9  | Prob. | 0.97 | 0.92 | 0.88 | 0.99 | 0.77 |
|                                         |         | Pred. | +    | +    | +    | +    | +    |
|                                         | CYP2D6  | Prob. | 0.58 | 0.77 | 0.54 | 0.81 | 0.99 |
|                                         |         | Pred. | –    | –    | –    | –    | –    |
|                                         | CYP3A4  | Prob. | 0.87 | 0.81 | 0.80 | 0.89 | 0.85 |
|                                         |         | Pred. | –    | –    | –    | +    | –    |
|                                         | CYP2E1  | Prob. | 0.98 | 0.95 | 0.96 | 0.99 | 0.79 |
|                                         |         | Pred. | –    | –    | –    | –    | –    |
|                                         |         | Prob. | 1.00 | 1.00 | 1.00 | 0.98 | 1.00 |
|                                         |         | Pred. | –    | –    | –    | –    | –    |

Note: “–” indicates negative; “+” indicates positive; TPSA—Topological polar surface area; WLOGP—Wildman–Crippen logP, used to calculate a molecule’s lipophilicity, represented by the partition coefficient between *n*-octanol and water; GI—gastrointestinal; BBB—blood–brain barrier; P-gp—P-glycoprotein; LD<sub>50</sub>—Median Lethal Dose amount of a substance required to kill 50% of a test population, expressed in milligrams per kilogram (mg/kg) of body weight; Toxicity classes and their corresponding colors are presented according to ProTox-3.0, following the globally harmonized system for the classification and labeling of chemicals (GHS); Pred.—prediction; Prob.—probability; AhR—Aryl hydrocarbon Receptor; AR—Androgen Receptor; AR-LBD—Androgen Receptor Ligand Binding Domain; ER $\alpha$ —Estrogen Receptor Alpha; ER-LBD—Estrogen Receptor Ligand Binding Domain; PPAR- $\gamma$ —Peroxisome Proliferator Activated Receptor Gamma; Nrf2/ARE—Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element; HSE—Heat shock factor response element; MMP—Mitochondrial Membrane Potential; p53—Phosphoprotein (Tumor Suppressor); ATAD5—ATPase family AAA domain containing protein 5; THR $\alpha$ —Thyroid hormone receptor alpha; THR $\beta$ —Thyroid hormone receptor beta; TTR—Transthyretin; RYR—Ryanodine receptor; GABAR—GABA receptor; NMDAR—Glutamate N-methyl-D-aspartate receptor; AMPAR—alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor; KAR—Kainate receptor; AChE—Acetylcholinesterase; CAR—Constitutive androstane receptor; PXR—Pregnane X receptor; NADHOX—NADH-quinone oxidoreductase; VGSC—Voltage gated sodium channel; NIS—Na<sup>+</sup>/I<sup>−</sup>symporter; CYP—Cytochrome P450 enzymes involved in drug metabolism and toxicity in the liver.

## References

- Ahmad-Mansour, N.; Loubet, P.; Pouget, C.; Dunyach-Remy, C.; Sotto, A.; Lavigne, J.-P.; Molle, V. Staphylococcus Aureus Toxins: An Update on Their Pathogenic Properties and Potential Treatments. *Toxins* **2021**, *13*, 677. <https://doi.org/10.3390/toxins13100677>.
- Bencardino, D.; Amagliani, G.; Brandi, G. Carriage of Staphylococcus Aureus among Food Handlers: An Ongoing Challenge in Public Health. *Food Control* **2021**, *130*, 108362. <https://doi.org/10.1016/j.foodcont.2021.108362>.
- Kozajda, A.; Jeřak, K.; Kapsa, A. Airborne Staphylococcus Aureus in Different Environments—A Review. *Env. Sci. Pollut. Res.* **2019**, *26*, 34741–34753. <https://doi.org/10.1007/s11356-019-06557-1>.

4. Chen, H.; Zhang, J.; He, Y.; Lv, Z.; Liang, Z.; Chen, J.; Li, P.; Liu, J.; Yang, H.; Tao, A.; et al. Exploring the Role of *Staphylococcus Aureus* in Inflammatory Diseases. *Toxins* **2022**, *14*, 464. <https://doi.org/10.3390/toxins14070464>.
5. Léguillier, V.; Pinamonti, D.; Chang, C.-M.; Gunjan; Mukherjee, R.; Himanshu; Cossetini, A.; Manzano, M.; Anba-Mondoloni, J.; Malet-Villemagne, J.; et al. A Review and Meta-Analysis of *Staphylococcus Aureus* Prevalence in Foods. *Microbe* **2024**, *4*, 100131. <https://doi.org/10.1016/j.microb.2024.100131>.
6. Da Silva, A.C.; Rodrigues, M.X.; Silva, N.C.C. Methicillin-Resistant *Staphylococcus Aureus* in Food and the Prevalence in Brazil: A Review. *Braz. J. Microbiol.* **2020**, *51*, 347–356. <https://doi.org/10.1007/s42770-019-00168-1>.
7. European Food Safety Authority (EFSA); European Centre for Disease Prevention and Control (ECDC). The European Union One Health 2024 Zoonoses Report. *EFSA J.* **2025**, *23*, e9759. <https://doi.org/10.2903/j.efsa.2025.9759>.
8. Abdallah, E.M.; Alhatlani, B.Y.; De Paula Menezes, R.; Martins, C.H.G. Back to Nature: Medicinal Plants as Promising Sources for Antibacterial Drugs in the Post-Antibiotic Era. *Plants* **2023**, *12*, 3077. <https://doi.org/10.3390/plants12173077>.
9. Semerdjieva, I.; Zheljazkov, V.D.; Radoukova, T.; Radanović, D.; Marković, T.; Dincheva, I.; Stoyanova, A.; Astatkie, T.; Kačániová, M. Essential Oil Yield, Composition, Bioactivity and Leaf Morphology of *Juniperus oxycedrus* L. from Bulgaria and Serbia. *Biochem. Syst. Ecol.* **2019**, *84*, 55–63. <https://doi.org/10.1016/j.bse.2019.04.001>.
10. Semerdjieva, I.B.; Zheljazkov, V.D.; Dincheva, I.; Astatkie, T.; Kačániová, M. Chemotypes of *Juniperus oxycedrus* in Bulgaria and the Antimicrobial Activity of Galbuli Essential Oils. *Ind. Crops Prod.* **2020**, *158*, 113005. <https://doi.org/10.1016/j.indcrop.2020.113005>.
11. Mërtiri, I.; Păcularu-Burada, B.; Stănciuc, N. Phytochemical Characterization and Antibacterial Activity of Albanian *Juniperus communis* and *Juniperus oxycedrus* Berries and Needle Leaves Extracts. *Antioxidants* **2024**, *13*, 345. <https://doi.org/10.3390/antiox13030345>.
12. Spengler, G.; Gajdács, M.; Donadu, M.G.; Usai, M.; Marchetti, M.; Ferrari, M.; Mazzarello, V.; Zanetti, S.; Nagy, F.; Kovács, R. Evaluation of the Antimicrobial and Antivirulent Potential of Essential Oils Isolated from *Juniperus oxycedrus* L. ssp. *Macrocarpa* Aerial Parts. *Microorganisms* **2022**, *10*, 758. <https://doi.org/10.3390/microorganisms10040758>.
13. Ertürk, Ö. Antibacterial and Antifungal Activity of Ethanolic Extracts from Eleven Spice Plants. *Biologia* **2006**, *61*, 275–278. <https://doi.org/10.2478/s11756-006-0050-8>.
14. Moumou, M.; Mokhtari, I.; Tayebi, A.; Milenkovic, D.; Amrani, S.; Harnafi, H. *Juniperus oxycedrus* L. Fruit, Leaves, and Essential Oil: A Systematic Literature Review on Bioactive Compounds, Pharmacological Properties and Toxicology. *Phytochem. Rev.* **2025**, *24*, 4759–4789. <https://doi.org/10.1007/s11101-024-10054-w>.
15. Fotiadou, E.; Panou, E.; Graikou, K.; Sakellarakis, F.-N.; Chinou, I. Volatiles of All Native *Juniperus* Species Growing in Greece—Antimicrobial Properties. *Foods* **2023**, *12*, 3506. <https://doi.org/10.3390/foods12183506>.
16. Mërtiri, I.; Mihalcea, L.; Aprodu, I.; Cioromila, A.C.; Turturică, M.; Râpeanu, G.; Stănciuc, N.; Grigore-Gurgu, L. Phytochemical Profile and Antilisterial Activity of *Salvia Officinalis* Supercritical Fluid Extract. *Appl. Food Res.* **2025**, *5*, 101379. <https://doi.org/10.1016/j.afres.2025.101379>.
17. Cvitković, D.; Škarica, I.; Dragović-Uzelac, V.; Balbino, S. Supercritical CO<sub>2</sub> Extraction of Fatty Acids, Phytosterols, and Volatiles from Myrtle (*Myrtus communis* L.) Fruit. *Molecules* **2024**, *29*, 1755. <https://doi.org/10.3390/molecules29081755>.
18. Dashtian, K.; Kamalabadi, M.; Ghoorchian, A.; Ganjali, M.R.; Rahimi-Nasrabadi, M. Integrated Supercritical Fluid Extraction of Essential Oils. *J. Chromatogr. A* **2024**, *1733*, 465240. <https://doi.org/10.1016/j.chroma.2024.465240>.
19. El Ahmadi, K.; El Allaoui, H.; El Abdouni, A.; Bouhrim, M.; Eto, B.; Dira, I.; Shahat, A.A.; Herqash, R.N.; Haboubi, K.; El Bas-trioui, M.; et al. A Bibliometric Analysis of the Supercritical CO<sub>2</sub> Extraction of Essential Oils from Aromatic and Medicinal Plants: Trends and Perspectives. *Horticulturae* **2024**, *10*, 1185. <https://doi.org/10.3390/horticulturae10111185>.
20. Marongiu, B.; Porcedda, S.; Piras, A.; Sanna, G.; Murreddu, M.; Loddo, R. Extraction of *Juniperus communis* L. ssp. *Nana* Willd. Essential Oil by Supercritical Carbon Dioxide. *Flavour. Fragr. J.* **2006**, *21*, 148–154. <https://doi.org/10.1002/ffj.1549>.
21. Orav, A.; Koel, M.; Kailas, T.; Müürisepp, M. Comparative Analysis of the Composition of Essential Oils and Supercritical Carbon Dioxide Extracts from the Berries and Needles of Estonian Juniper (*Juniperus communis* L.). *Procedia Chem.* **2010**, *2*, 161–167. <https://doi.org/10.1016/j.proche.2009.12.023>.
22. Aliev, A.M.; Radjabov, G.K.; Stepanov, G.V. Composition of Extract of the *Juniperus oblonga* M. Bieb. Fruits Obtained by Super-critical CO<sub>2</sub> Extraction. *Russ. J. Phys. Chem. B* **2013**, *7*, 795–801. <https://doi.org/10.1134/S1990793113070038>.
23. Floegel, A.; Kim, D.-O.; Chung, S.-J.; Koo, S.I.; Chun, O.K. Comparison of ABTS/DPPH Assays to Measure Antioxidant Capacity in Popular Antioxidant-Rich US Foods. *J. Food Compos. Anal.* **2011**, *24*, 1043–1048. <https://doi.org/10.1016/j.jfca.2011.01.008>.

24. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Hawkins Byrne, D. Comparison of ABTS, DPPH, FRAP, and ORAC Assays for Estimating Antioxidant Activity from Guava Fruit Extracts. *J. Food Compos. Anal.* **2006**, *19*, 669–675. <https://doi.org/10.1016/j.jfca.2006.01.003>.
25. Mrid, R.B.; Bouchmaa, N.; Bouargalne, Y.; Ramdan, B.; Karrouchi, K.; Kabach, I.; Karbane, M.E.; Idir, A.; Zyad, A.; Nhiri, M. Phytochemical Characterization, Antioxidant and In Vitro Cytotoxic Activity Evaluation of *Juniperus oxycedrus* subsp. *oxycedrus* Needles and Berries. *Molecules* **2019**, *24*, 502.
26. Kaseke, T.; Opara, U.L.; Fawole, O.A. Fatty Acid Composition, Bioactive Phytochemicals, Antioxidant Properties and Oxidative Stability of Edible Fruit Seed Oil: Effect of Preharvest and Processing Factors. *Heliyon* **2020**, *6*, e04962. <https://doi.org/10.1016/j.heliyon.2020.e04962>.
27. European Committee on Antimicrobial Susceptibility Testing (EUCAST). *EUCAST Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 16.0*; European Committee on Antimicrobial Susceptibility Testing: Växjö, Sweden, 2026.
28. Ishak, A.; Mazonakis, N.; Spernovasilis, N.; Akinosoglou, K.; Tsioutis, C. Bactericidal versus Bacteriostatic Antibacterials: Clinical Significance, Differences and Synergistic Potential in Clinical Practice. *J. Antimicrob. Chemother.* **2024**, *80*, 1–17. <https://doi.org/10.1093/jac/dkac380>.
29. Jeong, J.-Y.; Jung, I.-G.; Yum, S.-H.; Hwang, Y.-J. In Vitro Synergistic Inhibitory Effects of Plant Extract Combinations on Bacterial Growth of Methicillin-Resistant *Staphylococcus Aureus*. *Pharmaceuticals* **2023**, *16*, 1491. <https://doi.org/10.3390/ph16101491>.
30. Daina, A.; Zoete, V. A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. *ChemMedChem* **2016**, *11*, 1117–1121. <https://doi.org/10.1002/cmdc.201600182>.
31. Nguyen, T.-T.-L.; Duong, V.-A.; Maeng, H.-J. Pharmaceutical Formulations with P-Glycoprotein Inhibitory Effect as Promising Approaches for Enhancing Oral Drug Absorption and Bioavailability. *Pharmaceutics* **2021**, *13*, 1103. <https://doi.org/10.3390/pharmaceutics13071103>.
32. Bento-Silva, A.; Koistinen, V.M.; Mena, P.; Bronze, M.R.; Hanhineva, K.; Sahlström, S.; Kitrytė, V.; Moco, S.; Aura, A.-M. Factors Affecting Intake, Metabolism and Health Benefits of Phenolic Acids: Do We Understand Individual Variability? *Eur. J. Nutr.* **2020**, *59*, 1275–1293. <https://doi.org/10.1007/s00394-019-01987-6>.
33. Sharma, E.; Attri, D.C.; Sati, P.; Dhyani, P.; Szopa, A.; Sharifi-Rad, J.; Hano, C.; Calina, D.; Cho, W.C. Recent Updates on Anti-cancer Mechanisms of Polyphenols. *Front. Cell Dev. Biol.* **2022**, *10*, 1005910. <https://doi.org/10.3389/fcell.2022.1005910>.
34. Cadena-Iñiguez, J.; Santiago-Osorio, E.; Sánchez-Flores, N.; Salazar-Aguilar, S.; Soto-Hernández, R.M.; Riviello-Flores, M.d.l.L.; Macías-Zaragoza, V.M.; Aguiñiga-Sánchez, I. The Cancer-Protective Potential of Protocatechuic Acid: A Narrative Review. *Molecules* **2024**, *29*, 1439. <https://doi.org/10.3390/molecules29071439>.
35. Mahwish; Imran, M.; Naeem, H.; Hussain, M.; Alsagaby, S.A.; Al Abdulmonem, W.; Mujtaba, A.; Abdelgawad, M.A.; Ghoneim, M.M.; El-Ghorab, A.H.; Selim, S.; et al. Antioxidative and Anticancer Potential of Luteolin: A Comprehensive Approach Against Wide Range of Human Malignancies. *Food Sci. Nutr.* **2025**, *13*, e4682. <https://doi.org/10.1002/fsn3.4682>.
36. Wu, P.-S.; Yen, J.-H.; Kou, M.-C.; Wu, M.-J. Luteolin and Apigenin Attenuate 4-Hydroxy-2-Nonenal-Mediated Cell Death through Modulation of UPR, Nrf2-ARE and MAPK Pathways in PC12 Cells. *PLoS ONE* **2015**, *10*, e0130599. <https://doi.org/10.1371/journal.pone.0130599>.
37. Sirotkin, A.V.; Harrath, A.H. Apigenin as a Promising Agent for Enhancing Female Reproductive Function and Treating Associated Disorders. *Biomedicines* **2024**, *12*, 2405. <https://doi.org/10.3390/biomedicines12102405>.
38. da Rocha, G.H.O.; Müller, C.; Przybylski-Wartner, S.; Schaller, H.; Riemschneider, S.; Lehmann, J. AhR-Induced Anti-Inflammatory Effects on a Caco-2/THP-1 Co-Culture Model of Intestinal Inflammation Are Mediated by PPAR $\gamma$ . *Int. J. Mol. Sci.* **2024**, *25*, 13072. <https://doi.org/10.3390/ijms252313072>.
39. Changizi, Z.; Kajbaf, F.; Moslehi, A. An Overview of the Role of Peroxisome Proliferator-Activated Receptors in Liver Diseases. *J. Clin. Transl. Hepatol.* **2023**, *11*, 1542–1552. <https://doi.org/10.14218/JCTH.2023.00334>.
40. Chen, P.; Li, B.; Ou-Yang, L. Role of Estrogen Receptors in Health and Disease. *Front Endocrinol* **2022**, *13*, 839005. <https://doi.org/10.3389/fendo.2022.839005>.
41. Kim, M.; Jee, S.-C.; Sung, J.-S. Hepatoprotective Effects of Flavonoids against Benzo[a]Pyrene-Induced Oxidative Liver Damage along Its Metabolic Pathways. *Antioxidants* **2024**, *13*, 180. <https://doi.org/10.3390/antiox13020180>.
42. Křížková, J.; Burdová, K.; Stiborová, M.; Křen, V.; Hodek, P. The Effects of Selected Flavonoids on Cytochromes P450 in Rat Liver and Small Intestine. *Interdiscip. Toxicol.* **2009**, *2*, 201–204. <https://doi.org/10.2478/v10102-009-0018-y>.
43. Živić, N.; Milošević, S.; Dekić, V.; Dekić, B.; Ristić, N.; Ristić, M.; Srećić, L. Phytochemical and Antioxidant Screening of Some Extracts of *Juniperus communis* L. and *Juniperus oxycedrus* L. *Czech J. Food Sci.* **2019**, *37*, 351–358. <https://doi.org/10.17221/28/2019-CJFS>.

44. Zlatanović, I.; Stanković, M.; Ickovski, J.; Dimitrijević, I.; Stojanović, G. Comprehensive Analysis of the Herbal Mixture Made of *Juniperus oxycedrus* L. Berries, Inner Bark of *Betula pendula* Roth., and Grains of *Avena sativa* L. *Nat. Prod. Commun.* **2022**, *17*, 1934578X221105689. <https://doi.org/10.1177/1934578X221105689>.
45. Kachmar, M.R.; Majdoub, Y.O.E.; Oliveira, A.P.; Bouymajane, A.; Mrabti, H.N.; Bouddine, T.; Mir, N.; Mrabti, N.N.; Lhoussain, H.; Haloui, Z.; et al. *Juniperus oxycedrus* Leaves and Berries Extracts: HPLC-PDA-ESI/MS2 Phenolic Characterization and in Vitro Anti-Inflammatory Effects. *Phytomedicine Plus* **2024**, *4*, 100528. <https://doi.org/10.1016/j.phyplu.2024.100528>.
46. Olech, M.; Nowak, R.; Ivanova, D.; Tashev, A.; Boyadzhieva, S.; Kalotova, G.; Angelov, G.; Gawlik-Dziki, U. LC-ESI-MS/MS-MRM Profiling of Polyphenols and Antioxidant Activity Evaluation of Junipers of Different Origin. *Appl. Sci.* **2020**, *10*, 8921. <https://doi.org/10.3390/app10248921>.
47. De Rossi, L.; Rocchetti, G.; Lucini, L.; Rebecchi, A. Antimicrobial Potential of Polyphenols: Mechanisms of Action and Microbial Responses—A Narrative Review. *Antioxidants* **2025**, *14*, 200. <https://doi.org/10.3390/antiox14020200>.
48. Zhou, H.; Chen, L.; Ouyang, K.; Zhang, Q.; Wang, W. Antibacterial Activity and Mechanism of Flavonoids from *Chimonanthus salicifolius* S. Y. Hu. and Its Transcriptome Analysis against *Staphylococcus aureus*. *Front. Microbiol.* **2023**, *13*, 1103476. <https://doi.org/10.3389/fmicb.2022.1103476>.
49. Mandal, M.K.; Domb, A.J. Antimicrobial Activities of Natural Bioactive Polyphenols. *Pharmaceutics* **2024**, *16*, 718. <https://doi.org/10.3390/pharmaceutics16060718>.
50. Larkeche, O.; Zermane, A.; Meniai, A.-H.; Crampon, C.; Badens, E. Supercritical Extraction of Essential Oil from *Juniperus communis* L. Needles: Application of Response Surface Methodology. *J. Supercrit. Fluids* **2015**, *99*, 8–14. <https://doi.org/10.1016/j.supflu.2015.01.026>.
51. Balan, N.; Măntăilă, S.; Răpeanu, G.; Stănciuc, N. Enhanced Extraction of Bioactive Compounds from Red Grape Pomace: Optimizing Ultrasound-Assisted Extraction with Ethanol and NaDES as Solvents. *Antioxidants* **2025**, *14*, 526. <https://doi.org/10.3390/antiox14050526>.
52. ISO 20776-1:2019; Susceptibility Testing of Infectious Agents and Evaluation of Performance of Antimicrobial Susceptibility Test Devices. Part 1: Broth Micro-Dilution Reference Method for Testing the In Vitro Activity of Antimicrobial Agents Against Rapidly Growing Aerobic Bacteria Involved in Infectious Diseases (Edition 2, 2019); International Standardization Organization: Geneva, Switzerland, 2019.
53. Gómez-Sequeda, N.; Cáceres, M.; Stashenko, E.E.; Hidalgo, W.; Ortiz, C. Antimicrobial and Antibiofilm Activities of Essential Oils against *Escherichia Coli* O157:H7 and Methicillin-Resistant *Staphylococcus Aureus* (MRSA). *Antibiotics* **2020**, *9*, 730. <https://doi.org/10.3390/antibiotics9110730>.
54. Cai, Y.; Zou, G.; Xi, M.; Hou, Y.; Shen, H.; Ao, J.; Li, M.; Wang, J.; Luo, A. Juglone Inhibits *Listeria Monocytogenes* ATCC 19115 by Targeting Cell Membrane and Protein. *Foods* **2022**, *11*, 2558. <https://doi.org/10.3390/foods11172558>.
55. Martínez, A.; Manrique-Moreno, M.; Klaiss-Luna, M.C.; Stashenko, E.; Zafra, G.; Ortiz, C. Effect of Essential Oils on Growth Inhibition, Biofilm Formation and Membrane Integrity of *Escherichia Coli* and *Staphylococcus Aureus*. *Antibiotics* **2021**, *10*, 1474. <https://doi.org/10.3390/antibiotics10121474>.
56. Güven, L.; Hancı, H. GC–MS Profile, Antimicrobial Activity, and In Silico ADMET Evaluation of Major Constituents from *Pogostemon cablin* (Patchouli) and *Juniperus communis* (Juniper). *Curr. Res. Health Sci.* **2025**, *2*, 111–122. <https://doi.org/10.62425/crhis.1776763>.

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