Natural Compounds in *Pinus pinaster* Needles Essential Oil as an Antibacterial Agent: In Vitro and in Silico Studies

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Abstract: - Recently, there has been growing interest in the antimicrobial potential of natural compounds found in essential oils (EOs). In this study, the antibacterial activity of *Pinus pinaster* essential oil (EO) was assessed against both Gram-negative and Gram-positive bacterial species using the microdilution method in microplates. Additionally, 18 natural compounds from this EO were evaluated through molecular docking for their inhibitory properties on PBP1, PBP2, and PBP3—crucial enzymes in the development of novel antibiotics. The DFT properties, drug-likeness, and ADMET predictions of these compounds were also investigated. The results demonstrated moderate antibacterial activity of P. pinaster EO against all tested bacterial strains, with inhibition zones ranging from 11 to 16 mm. Molecular docking studies indicated that Terpinen-7-al was the most potent compound, binding to the highest number of amino acids within the active sites of the target enzymes, as suggested by the native ligand of each enzyme. Furthermore, the DFT study, along with ADMET and drug-likeness analysis, identified Terpinen-7-al as the most promising compound. Therefore, *P. pinaster* EO could serve as a significant source of natural molecules with promising antibacterial properties, particularly Terpinen-7-al, which has the potential for development into an antibiotic treatment.

Key-Words: - essential oil, antibacterial, biomedicine, inhibition, PBPs molecular docking, ADMET.

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1 Introduction

The rise of multi-drug resistant (MDR) bacteria poses a significant challenge to modern medicine fueled in part by the continuous and often indiscriminate use of antibiotics, [1] . Over the past century, antibiotics have revolutionized healthcare, saving countless lives by effectively treating bacterial infections, [2] .

However, their widespread use and sometimes misuse have inadvertently provided a breeding ground for bacteria to develop resistance mechanisms, rendering many antibiotics ineffective, [3] . In this context, the search for new antimicrobial agents and innovative treatment strategies is crucial for addressing the evolving threat posed by MDR bacteria and ensuring continued access to effective therapies for infectious diseases, [4] .

Moreover, research efforts aimed at understanding the mechanisms of antibiotic resistance and developing strategies to combat it are critical for preserving the efficacy of existing antibiotics and prolonging their lifespans, [5] . In this regard, antibacterial essential oils (EOs) have garnered considerable attention as a promising source for discovering new antibacterial drugs, [6] .

These oils, derived from plants, contain a diverse array of bioactive compounds that have demonstrated antimicrobial properties against a wide range of bacterial pathogens. Therefore, exploring the therapeutic potential of antibacterial EOs represents a promising avenue for combating antibiotic-resistant infections and developing novel treatment strategies, [7] . In the realm of drug discovery and development, the quest for effective therapeutic agents hinges on the ability to accurately predict how molecules interact with target proteins. Molecular docking is a powerful method for assessing drug activity against specific protein targets, [8] .

Targeting proteins like penicillin-binding proteins (PBPs) is crucial for developing new antibiotics to fight bacterial infections, [9] . PBPs are essential for bacterial cell wall synthesis; inhibiting them disrupts peptidoglycan assembly, causing cell lysis and death, [10] .

This study aims to evaluate the antibacterial activity of *P. pinaster* EO against Gram-negative and Gram-positive bacteria and identify the EO's most promising natural compounds that inhibit key bacterial enzymes (PBP1, PBP2, and PBP3).

2 Methodology

2.1 Antibacterial Activity

Pinus pinaster needles EO was evaluated against several Gram-negative (*Escherichia coli* ATCC 7839) and Gram-positive (*Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13883 and *Staphylococcus aureus* ATCC BAA-2856) bacterial strains.

2.2 Molecular Docking Studies

The proteins analyzed in this study were downloaded from the RCSB Protein Data Bank, [11] They include PBP1 of *S. aureus* (PDB ID: 7O4B), PBP2 of *E. coli* (PDB ID: 6G9S), and PBP3 of *K. pneumoniae* (PDB ID: 8GPW), with resolutions of 2.00, 1.59, and 2.59 Å, respectively.

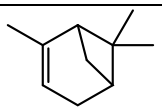
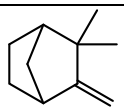
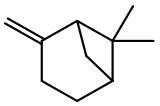
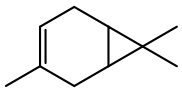
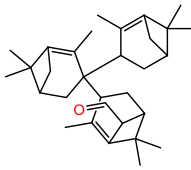
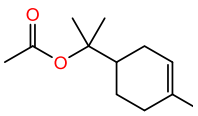
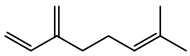
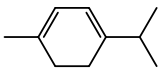
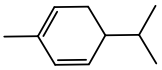
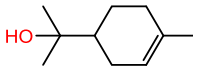
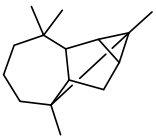
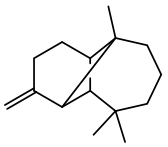
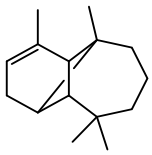
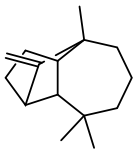
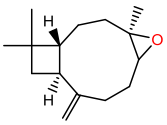
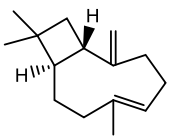
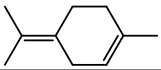
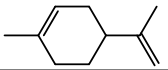
Molecular docking was executed using AMDock v1.5.2, [12] . Integrated with AutoDock Vina 1.2.1, [13] and the protein pH was maintained at 7.4.

The validation of the docking protocol involved comparing the docked position of a co-crystallized ligand to its original configuration within the target protein. This comparison was done using PyMOL 1.8.5, [14] , embedded in AMDock, using root mean square deviation (RMSD) calculations. Lower RMSD values, especially those under 2.0 Å, indicate greater accuracy of the docking protocol. [15] , grid boxes were established in AMDock using the "Center on Ligand" option, which places a box with optimal dimensions at the ligand's geometric center. All molecular interactions were visualized using BIOVIA Discovery Studio Visualizer in both 3D and 2D formats, [16] .

As ligands, natural compounds were derived from previous studies investigating the HPLC profiles of *P. pinaster* needles EO, [17] , [18] .

The chemical structures of these compounds are shown in Table 1.

Table 1. Main natural compounds from *P. pinaster* needles EO

Compound	Chemical structure	Compound	Chemical structure
α-Pinene		Camphene	
β-Pinene		3-Carene	
Terpinen-7-al		α-terpinyle Acetate	
Myrcene		α-Terpinene	
α-Phellandrene		α-Terpineol	
Longicyclene		β-Longipinene	
α-Longipinene		Longifolene	
Caryophyllene oxide		Caryophyllene	
Terpinolene		Limonene	

2.3 Density Functional Theory

To predict the properties of the compounds, Density Functional Theory (DFT) calculations were performed using Gaussian 09, **Σφάλμα! Το αρχείο προέλευσης της αναφοράς δεν βρέθηκε.** and visualized with Gauss View 6.0.16, [20] . Geometry optimizations were done with the B3LYP/6-31G(d) basis set for the most promising compounds in terms of interaction energies (ΔG).

Stability and reactivity were assessed by calculating various descriptors, including HOMO and LUMO, energy gap (ΔE_{gap}), ionization potential (I), chemical hardness (η), chemical softness (σ), and dipole moment (μ), [21] .

2.4 Drug-likeness and ADMET Analysis

SwissADME webserver, [22] , was used to predict the drug-likeness, pharmacokinetic, and

toxicological parameters of the tested compounds, including rotatable bonds, bioavailability score, TPSA, water solubility, lipophilicity, GI absorption, BBB penetration, skin permeation, P-gp substrate prediction, and Cytochrome P450 enzyme inhibition.

ProTOX III webserver, [23] , calculated LD50 values, toxicity classes, and organ and endpoint toxicities.

3 Results and Discussion

The antibiogram is a test that determines the sensitivity of bacteria to various antibiotics. The results are expressed in terms of the diameter of the inhibition zone, which indicates the ability of the antibiotic to inhibit bacterial growth, [24] .

As illustrated in Figure 1 and detailed in Table 2, the results indicate that amoxicillin (AMX120) is effective against *S. typhimurium* (24 mm) and *S. aureus* (21 mm), showing relatively large inhibition zones, while against *E. coli* (16 mm), the effectiveness was moderate. On the other hand, *K. pneumoniae* showed complete resistance (0 mm), meaning that amoxicillin is not effective against this bacterium. Regarding cefazolin (CZ 30), the results show effectiveness against *S. typhimurium* (21 mm), *S. aureus* (20 mm), and particularly against *K. pneumoniae* (23 mm). Against *E. coli* (15 mm), the effectiveness is comparable to that of amoxicillin.

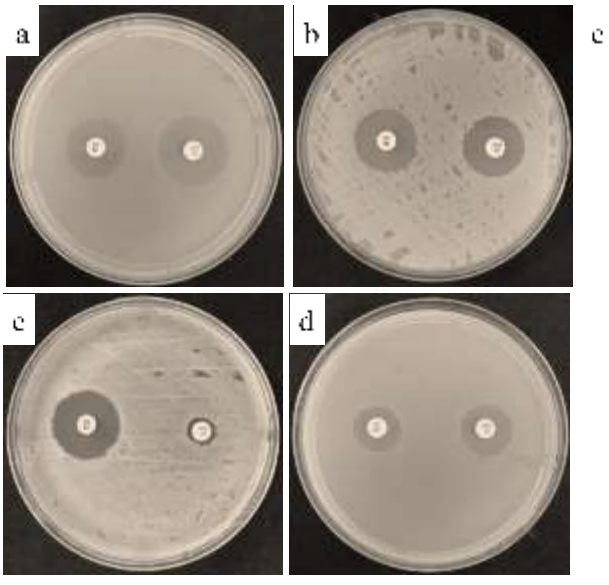


Fig. 1: Reference antibiogram of cefazolin (left) and amoxicillin (right). a) *S. typhimurium*, b) *S. aureus* c) *K. pneumoniae*, d) *E. coli*

The aromatogram is a technique similar to the antibiogram, but it uses EOs to test the sensitivity of bacteria, [25] . The results obtained from the aromatogram of *P. pinaster* against *S. typhimurium*, *S. aureus*, *K. pneumoniae*, and *E. coli* are displayed in Figure 2.

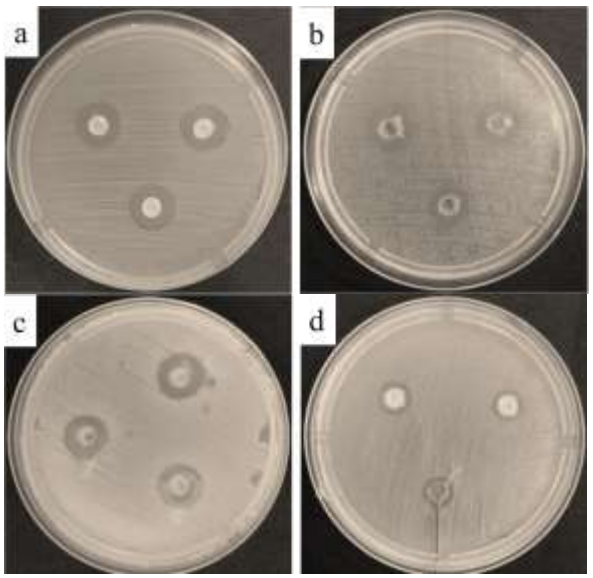


Fig. 2: Aromatogram of *P. pinaster* against the tested bacterial strains. a) *S. typhimurium*, b) *S. aureus*, c) *K. pneumoniae*, d) *E. coli*

As shown in Table 2. The EO of *P. pinaster* needles exhibited moderate antimicrobial activity against all the tested stains, with inhibition zones of 15 mm against *S. typhimurium*, 16 mm against *S. aureus*, 14 mm against *K. pneumoniae*, and 11 mm against *E. coli*. This activity is relatively modest compared to conventional antibiotics.

Table 2. Inhibition zone of reference antibiotics and *P. pinaster* EO

Inhibition zone (mm)	*S. typhimurium*	*S. aureus*	*K. pneumoniae*	*E. coli*
AMX120	*24* ± 1.65	*21* ± 1.14	-	*16* ± 0.79
CZ 30	21 ± 1.56	20 ± 1.54	23 ± 2.38	15 ± 0.66
P. pinaster needles oil	14.83 ± 0.29	16.16 ± 1.15	13,67 ± 0.58	10,8 ± 0.76

-: No activity found

3.1 Molecular Docking

RMSD is an essential measure in molecular docking. It allows for the evaluation of structural similarity between two molecular configurations, often between a reference experimental structure and a predicted structure, or between different conformations of the same molecule, [26] .

In our study, the RMSD values between the positions of the redocked ligands and the native ligands were 0.403 Å for 7O4B, 1.524 Å for 6G9S, and 0.879 Å for 8GPW. These values are below the acceptable threshold of 2 Å. As shown in Figure 3, there is a good overlap between the pose simulated

by Autodock Vina (colored in blue) and the native ligand (colored in red).

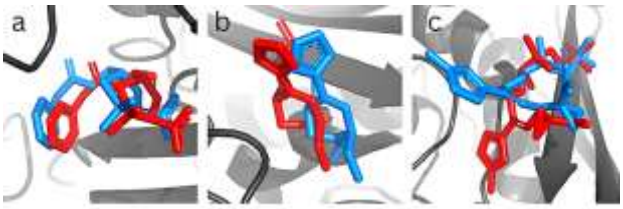


Fig. 3: Superimposition of the docked (blue) and native (red) co-crystallized complexes. a) PBP1 of *S. aureus* (7O4B), b) PBP2 of *E. coli* (6G9S), c) PBP3 of k. pneumonia (8GPW).

The results obtained from the molecular docking of each compound from *P. pinaster* EO with PBP1 of *S. aureus* (7O4B), are displayed in Table 3.

Table 3. Binding energies of natural compounds from *P. pinaster* EO against PBP1

Compound		**PBP1 of *S. aureus* (7O4B)**	
		ΔG kJ mol^{-1}	*Ki* µM
Ref	Amoxicillin	-33.472	1.37
	Cefazolin	-37.238	299.41
α-Pinene		-20.50	2600
β-Pinene		-20.50	2600
Camphene		-20.08	3000
Myrcene		-19.25	4200
α-Phellandrene		-19.66	3600
Δ-3-Carene		-22.59	1100
α-Terpinene		-21.34	1800
Limonene		-20.50	2600
Terpinolene		-21.76	1500
α-Terpineol		-22.17	1300
Terpinen-7-al		-39.75	1.80
α-Terpinyl Acetate		-26.78	20.36
α-Longipinene		-27.61	14.53
Longicyclene		-27.20	17.20
β-Longipinene		-25.52	33.78
Longifolene		-28.45	10.37
Caryophyllene		-26.78	20.36
Caryophyllene oxide		-27.20	17.20

The results obtained from the molecular docking of each compound from *P. pinaster* EO with PBP2 of *E. coli* (6G9S), are displayed in Table 4.

Table 4. Binding energies of natural compounds from *P. pinaster* EO against PBP2

Compound		**PBP2 of *E. coli* (6G9S)**	
		ΔG kJ mol^{-1}	*Ki* µM
Ref	Amoxicillin	-7.5	3.18
	Cefazolin	-8.0	1.37
α-Pinene		-19.66	3600
β-Pinene		-19.66	2600
Camphene		-19.25	4200
Myrcene		-18.83	5000
α-Phellandrene		-18.83	5000
Δ-3-Carene		-21.34	1800
α-Terpinene		-21.34	1800
Limonene		-18.83	5000
Terpinolene		-21.34	1800
α-Terpineol		-20.92	2200
Terpinen-7-al		-30.54	4.46
α-Terpinyl Acetate		-25.52	33.78
α-Longipinene		-23.85	66.36
Longicyclene		-24.27	56.05
β-Longipinene		-23.85	66.36
Longifolene		-24.69	47.35
Caryophyllene		-23.85	33.36
Caryophyllene oxide		-28.45	10.37

The results obtained from the molecular docking of each compound from *P. pinaste*r with EO PBP3 (8GPW) of *K. pneumoniae* are displayed in the Table 5.

Table 5. Binding energies of natural compounds from *P. pinaster* EO against PBP1

Compound		**PBP3 of *K. pneumoniae* (6R3X)**	
		ΔG kJ mol^{-1}	*Ki* µM
Ref	Amoxicillin	-7.8	1.62
	Cefazolin	-8.6	0.49
α-Pinene		-19.25	420
β-Pinene		-19.25	420
Camphene		-18.83	500
Myrcene		-17.57	420
α-Phellandrene		-20.50	260
Δ-3-Carene		-19.25	360
α-Terpinene		-21.34	260
Limonene		-21.76	150
Terpinolene		-21.34	220
α-Terpineol		-23.01	93.00
Terpinen-7-al		-35.15	0.67
α-Terpinyl Acetate		-25.94	28.53
α-Longipinene		-25.52	33.78
Longicyclene		-24.69	39.99
β-Longipinene		-25.10	39.99
Longifolene		-24.27	56.05
Caryophyllene		-25.94	28.53
Caryophyllene oxide		-27.61	14.53

ΔG represents the change in free energy during the interaction between the compounds (ligands) and the target protein. It aims to evaluate the potential binding between the ligand and the receptor by using energy functions and algorithms to quantitatively measure the affinity (binding affinity) between the two molecules, [27] .

Upon analyzing the molecular docking results, we observe that Terpinen-7-al exhibits the lowest ΔG values (-39.75, -30.54, and -39.75 kJ mol-1 against *S. aureus*, *E. coli*, and *K. pneumoniae*, respectively), making it the most effective among all the molecules that compose EO. Additionally, this molecule surpasses the effectiveness of the reference drugs (Amoxicillin and Cefazolin) in terms of ΔG for PBP1 of *S. aureus* (7O4B) and is more effective than Amoxicillin for PBP3 of *K. pneumoniae* (8GPW).

In terms of inhibition constants (Ki), Terpinen-7-al also has the lowest values (1.80, 4.46, and 0.67 µM for *S. aureus*, *E. coli*, and *K. pneumoniae*, respectively), indicating high efficacy in inhibiting the target protein's activity at low concentrations.

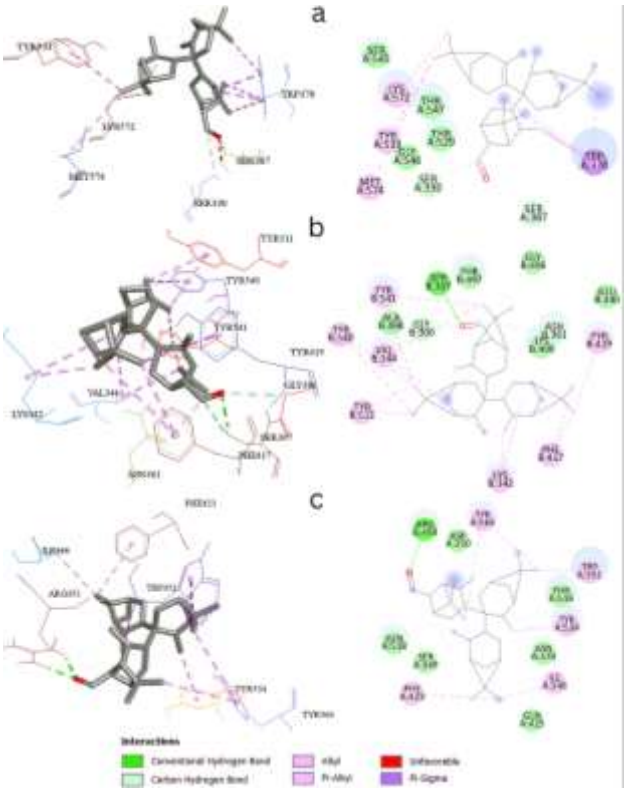


Fig. 4: The molecular interactions of Terpinen-7-al with a) PBP2 (6G9S), b) with PBP3 (8GPW), and c) with PBP1(7O4B)

The interactions of Terpinen-7-al with the selected proteins were investigated using Discovery Studio (Figure 4) With *E. coli*, Terpinen-7-al binds in the binding cavity, forming 2 hydrogen bonds and 8 hydrophobic interactions. The hydrogen bonds consist of 2 carbon-hydrogen (C-H) types with lengths of 3.45 Å and 3.51 Å, while the hydrophobic interactions range between 3.65 and 5.36 Å and include various types (two Pi-Sigma, two alkyl, and four Pi-Alkyl bonds). There is also an unfavourable bond (2.87 Å).

In the case of *K. pneumoniae*, Terpinen-7-al forms 4 hydrogen bonds, including one conventional hydrogen bond of 2.44 Å and three C-H bonds (3.02-3.80 Å). It also interacts through thirteen hydrophobic bonds, comprising four alkyl (3.93-5.13 Å) and nine Pi-Alkyl (4.32-5.42 Å).

Regarding *S. aureus*, the molecule forms two conventional hydrogen bonds (2.11 and 2.24 Å) and six hydrophobic interactions, including one alkyl (4.53 Å) and five Pi-Alkyl (3.91-5.23 Å). The interacting residues are presented in Figure 4.

3.2 Density Functional Theory

Determining the electronic parameters of molecules through the DFT is a powerful tool for predicting and explaining their reactivity in different reaction types and remains an efficient method for interpreting biological activity results, [28] .

Table 6. Electronic properties of *P. pinaster* EO natural compounds

Compound	Terpinen-7-al	Caryophyllene	Longicyclene	Longifolene
HOMO (eV)	-4.743	-5.950	-6.685	-6.244
LUMO (eV)	-0.972	0.529	2.106	0.697
E_{gap} (eV)	3.771	6.479	8.791	6.941
Ionization potential (I)	4.743	5.950	6.685	6.244
Chemical potential (µ)	-2.858	-2.710	-2.289	-2.773
Chemical hardness (η)	1.886	3.240	4.396	3.470
Chemical softness (S)	0.265	0.154	0.114	0.144
Dipole moment (D)	1.769	0.349	0.155	0.494

ΔE_{gap} of the compounds suggests varying degrees of stability, reactivity, and potential applications, [29] . According to DFT results displayed in Table 6, it appears that Terpinen-7-al, with the smallest gap (3.771 eV), is likely more reactive and could be useful in applications requiring high reactivity. Longicyclene, with the largest gap (8.791 eV), is the most stable and could be advantageous in applications where chemical stability is essential. Longicyclene, with the highest hardness (4.396 eV) and the lowest softness (0.114 eV), is the most stable and least reactive, making it suitable for applications where stability is required.

Terpinen-7-al, with the lowest hardness (1.886 eV), is the most reactive and least stable, which confirms the energy gap results.

The dipole moments provide valuable information about the polarity, solubility, and intermolecular interactions of the compounds, [30] . Terpinen-7-al, with the highest dipole moment (1.769 D), is the most polar and likely the most soluble in polar solvents, making it suitable for applications requiring high polarity. Longicyclene, with the lowest dipole moment 0.155 D), is the least polar.

3.3 Drug-likeness and ADMET Analysis

By analyzing the obtained results in Table 7, we observe that 50% of the compounds studied from the EO of *P. pinaster* (Myrcene, α-Phellandrene, α-Terpinene, Limonene, δ-Terpinene, Terpinolene, cis-β-Terpineol, α-Terpineol, α-Terpinyl Acetate, and Caryophyllene Oxide) meet the criteria of Lipinski's rule of five. The other molecules adhered to all the rules, except for Log Po/w (MLOGP), which exceeded 4.15. This high lipophilicity (log P > 4.15) may present challenges in terms of oral bioavailability, distribution within the body, and potential toxicity. Therefore, it is necessary to conduct further studies to evaluate their pharmacokinetic and pharmacodynamic properties in greater detail.

Table 7. Drug-likeness analysis of natural compounds from *P. pinaster* EO

Compound	*MW* g mol^{-1}	Violation of Lipinski rule
α-Pinene	136.23	No
β-Pinene	136.23	No
Camphene	136.23	No
Myrcene	136.23	Yes
α-Phellandrene	136.23	Yes
Δ-3-Carène	136.23	No
α-Terpinene	136.23	Yes
Limonene	136.23	Yes
Terpinolene	136.23	Yes
α-Terpineol	154.25	Yes
Terpinen-7-al	432.68	No
α-Terpinyl Acetate	196.29	Yes
α-Longipinene	204.35	No
Longicyclene	204.35	No
β-Longipinene	204.35	No
Longifolene	204.35	No
Caryophyllene	204.35	No
Caryophyllene oxide	220.35	Yes

Regarding the ADME analysis, the results shown in Table 8 indicate that most compounds exhibit good water solubility. Intestinal absorption was low for all compounds except cis-β-Terpineol, α-Terpineol, and α-Terpinyl Acetate, which demonstrated high absorption. The study revealed that most compounds from *P. pinaster* EO are capable of crossing the blood-brain barrier. However, compounds such as β-Caryophyllene Oxide, α-Longipinene, β-Longipinene, Longifolene, and Caryophyllene were found to be unable to cross this barrier. P-glycoprotein (P-gp) plays a critical role in the ADME of drugs by influencing their absorption, distribution, metabolism, and excretion. A thorough analysis of drug interactions with P-gp is essential for the development of new drugs, understanding their pharmacokinetics, and optimizing therapies, particularly concerning drug resistance and drug interactions, [31] . The results of this study (Table 8) show that no compound derived from *P. pinaster* affects this enzyme.

Table 8. ADMET properties of natural compounds from *P. pinaster* EO

Compound	GIA	Distribution		Metabolism for CYP450 inhibitor					Excretion Log ml min^{-1} kg^{-1}	DL_{50} mg kg^{-1}	Tox. Class	Tox End-point
		BBB	log *Kp*	1A2	2C19	2C9	2D6	3A4				
α-Pinene	Low	Yes	-3.95	No	No	Yes	No	No	0.043	3700	5	-
β-Pinene	Low	Yes	-4.18	No	No	Yes	No	No	0.030	4700	5	-
Camphene	Low	Yes	-4.13	No	No	Yes	No	No	0.049	5000	5	-
Myrcene	Low	Yes	-4.17	No	No	No	No	No	0.438	5000	5	-
α-Phellandrene	Low	Yes	-4.85	No	No	No	No	No	0.200	5700	6	-
Δ-3-Carene	Low	Yes	-4.97	No	No	No	No	No	0.037	4800	5	I.Tx
α-Terpinene	Low	Yes	-4.11	No	No	No	No	No	0.223	1680	4	-
Limonene	Low	Yes	-3.89	No	No	Yes	No	No	0.213	4400	5	-
Terpinolene	Low	Yes	-3.96	No	No	Yes	No	No	0.218	4390	5	-
α-Terpineol	High	Yes	-4.83	No	No	No	No	No	1.219	2830	5	-
Terpinen-7-al	Low	No	-4.84	No	No	No	No	No	-0.147	15000	6	-
α-Acetate terpinyle	High	Yes	-19.25	No	No	No	No	No	1.247	4800	5	H.Tx
α-Longipinene	Low	No	-4.27	No	Yes	Yes	No	No	0.863	3700	5	-
Longicyclene	Low	Yes	-3.99	Yes	Yes	Yes	No	No	0.680	15380	6	-
β-Longipinene	Low	No	-4.10	No	Yes	Yes	No	No	0.879	5000	5	-
Longifolene	Low	No	-3.94	No	Yes	Yes	No	No	0.901	5000	5	-
Caryophyllene	Low	No	-4.44	No	Yes	Yes	No	No	1.088	5300	5	I.Tx
Caryophyllene oxide	High	Yes	-5.12	No	Yes	Yes	No	No	0.905	5000	5	M.Gn

-: No endpoint toxicity; **I.Tx**: immunotoxicity; **H.Tx**: hepatotoxicity; **M.Gn**: mutagenicity; **C.Gn**: carcinogenicity

Cytochrome P450 (CYP450) enzymes are a crucial family involved in the metabolism of many drugs and endogenous substances. Interaction with these enzymes can lead to drug interactions or alter the rate at which a molecule is degraded and eliminated from the body, [32] . The results indicate that none of the studied compounds affect the CYP1A2 subfamily, except Longicyclene. Similarly, no compound affects CYP2C19, except α-Longipinene, Longicyclene, β-Longipinene, Longifolene, Caryophyllene, and Caryophyllene Oxide. Additionally, all compounds affect CYP2C9 except α-Phellandrene, Δ-3-Carene, α-Terpinene, δ-Terpinene, cis-β-Terpineol, α-Terpineol, Terpinen-7-al, and α-Terpinyl Acetate. It was noted that CYP2D6 and CYP3A4 are not affected by any compound.

In light of the obtained results, it is evident that the predicted LD50 values for the compounds of *P. pinaster* range from 1680 to 15380 mg kg-1. None of these compounds exhibited cytotoxicity or

carcinogenicity. However, α-Terpinyl Acetate was predicted to be hepatotoxic, and Caryophyllene Oxide was predicted to be mutagenic. Regarding immunotoxicity, only the compounds Δ-3-Carene and Caryophyllene were found to be active.

4 Conclusion

The essential oil of *Pinus pinaster* needles exhibits moderate antimicrobial activity against *S. typhimurium*, *S. aureus*, *K. pneumoniae*, and *E. coli*. Although this activity is less potent than that of traditional antibiotics, it may hold value in specific applications, particularly as a complement to existing treatments or in scenarios involving antibiotic resistance. Further research is necessary to better understand its clinical applications and to optimize its use.

The obtained *in silico* results indicate that Terpinen-7-al is a promising candidate for inhibiting PBPs due to: a stable and favorable interaction with the protein (low ΔG) and high efficacy at low concentrations (low Ki). These characteristics are encouraging for the potential development of Terpinen-7-al as a therapeutic inhibitor of PBPs.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process

During the preparation of this work the authors used ChatGPT in order to improve the readability and language of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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- Yazid Bedouh and Fares Hamoud, were responsible for project administration, writing original draft, review & editing, visualization, supervision, methodology, investigation, and conceptualization.
- Hani Belhadj was responsible for Methodology, Supervision, validation and visualization.
- Ismail Bennais, Radjaa Belatel, Anfel Belhaouam were in charge for software, formal analysis, methodology, resources, and data curation.

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