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In silico study of various compounds from essential oil of *Cymbopogon winterianus* against *Pseudomonas aeruginosa* targets

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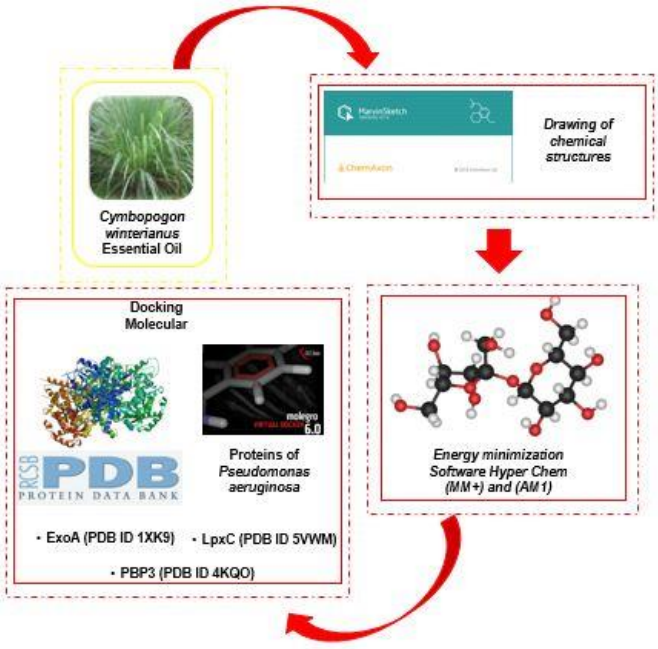
Graphical Abstract



Abstract

Pseudomonas aeruginosa is a gram-negative bacterium that can be found in soil, aquatic environments or on the surface of animals, plants and humans [1]. It is commonly isolated in immunocompromised patients in intensive care units and can cause urinary tract infections, pneumonia, folliculitis, otitis, keratitis, osteomyelitis and meningitis [2,3]. The compounds present in oils of species of the genus Cymbopogon are known to present several activities, including antimicrobial activity [4]. This work aims to perform a multi-target molecular modeling of essential oil components from citronella (Cymbopogon winterianus) against P. aeruginosa.

Materials and Methods

Initially, the 2D chemical structures of the 15 compounds under study were designed using ChemAxon's MarvinSketch 19.9 [5]. These structures were then imported into the software HyperChemTM 8.0.6 to optimize them using the molecular mechanics method (MM +) and the semi-empirical method (AM1)[6], where the number of cycles was adjusted to 600. Thus, the 3D structures of each molecule were obtained in the lowest energy conformation.

The three crystallographed proteins chosen as targets were exotoxin A (ExoA), UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase (LpxC) and penicillin-binding protein 3 (PBP3). Protein resolution values were 2.1 Å for ExoA (PDB ID 1XK9), 1.8 Å for LpxC (PDB ID 5VWM) and 2.31 Å for PBP3 (PDB ID 4KQO). All presented inhibitors coupled to their crystallographic structure, which allowed the delimitation of the active site of each protein.

Molecular docking was performed using the Molegro Virtual Docker 6.0 [7], using the molecules under study, antibacterial drugs used as controls and co-crystallized inhibitors with the three proteins. In order to classify the best molecules, the total energy of ligand-receptor interaction was verified, where the best value is the lowest [8].

The docking procedure was validated by re-docking the co-crystallized inhibitor in the active site of protein, thus, it is possible to estimate the Root Mean Square Deviation (RMSD)[9]. RMSD is calculated between the coordinates of the heavy atoms of the crystallographically determined ligand structure and the docked ligand [10]. RMSD < 2.0 Å is widely accepted in the literature for docking prediction [11].

The software Molegro also allows the analysis of ligand interactions with protein amino acid residues: hydrogen bonds (blue dashed lines), steric interactions (red dashed lines)[12] and electrostatic interactions (green dashed lines)[13]. Therefore, the types of interactions of 5 poses for each protein were obtained.

Results and Discussion

The molecular docking procedure was performed with the compounds under study where they demonstrated promising ligand-receptor interaction energies for all proteins. These energies ranged from -70.723 kcal.mol^{-1} to -972.659 kcal.mol^{-1} for ExoA, from -60.972 kcal.mol^{-1} to -772.392 kcal.mol^{-1} for LpxC and from -564.702 kcal.mol^{-1} to -900.617 kcal.mol^{-1} for PBP3, as shown in Table 1. RMSD results were: 0,289681 Å (ExoA), 0,345203 Å (LpxC) and 0,328067 Å (PBP3); indicating that the molecular docking procedure is reliable.

Table 1. Interaction energies [kcal.mol^{-1}] of the molecules and controls against *P. aeruginosa* targets.

Pose	ExoA	LpxC	PBP3
Citronellyl acetate	-972.659	-718.769	-775.039
Geranyl acetate	-70.723	-72.553	-721.124
α-Muurolene	-820.403	-602.152	-735.522
β-Citronellal	-866.123	-60.972	-668.695
β-Citronellol	-804.253	-617.498	-674.969
β-Elemene	-596.179	-532.445	-711.118
Δ-Cadinene	-732.812	-596.927	-633.604
Elemol	-585.343	-531.802	-757.164
γ-Cadinene	-838.816	-62.512	-698.176
γ-Muurolene	-838.704	-592.478	-698.416
Geranial	-792.246	-626.707	-678.962
Geraniol	-874.511	-646.654	-692.213
Germacrene D	-92.444	-772.392	-828.819
Germacrene D-4-ol	-784.573	-737.488	-900.617
Limonene	-769.529	-482.077	-564.702
Amikacin	-162.545	-160.819	-168.637
Aztreonam	-158.029	-130.684	-133.231
Cefepime	-144.012	-153.498	-156.612
Ceftazidime	-159.975	-154.172	-160.469
Ciprofloxacin	-110.095	-955.973	-982.283
Levofloxacin	-986.258	-754.054	-862.253
Meropenem	-131.028	-122.321	-137.896
Piperacillin	-148.243	-115.998	-178.562

It can be noted that the molecular docking results were excellent, since all molecules under study presented negative values of interaction energies with each protein. This indicates that they have a multi-target effect, which increases the likelihood of their biological activity.

The interactions between amino acid residues of each protein were also verified with: the best pose, the 3 compounds that showed the lowest energies among the molecules under study and with the inhibitors. For ExoA (Table 2), the levofloxacin control showed the lowest energy between poses and made hydrogen bonds with the residues Thr442, Glu553 and Tyr481. This latter residue also makes hydrogen bonds with citronellyl acetate and β-citronellal. Citronellyl acetate showed a very low interaction energy that approached the energy of levofloxacin, where it can be observed that these two molecules made steric interactions with the residues His440, Gly441 and Tyr481.

Table 2. Energy values and types of interactions between poses and amino acid residues of ExoA protein.

PDB ID	*Pose*	Energy [kcal.mol^{-1}]	Interactions	
			Types	Residues
1XK9	Levofloxacin	-986.258	H-bond Steric Electrostatic	Thr442, Tyr481 and Glu553 2(His440), 3(Gly441), Ile471, Ala478, 2(Leu477), Tyr481, 3(Glu553) and 2(Tyr470) None
1XK9	Citronellyl acetate	-972.659	H-bond Steric Electrostatic	Tyr481 2(Gly441), 2(Tyr470), 2(Tyr 481), Tyr 439 and His440 None
1XK9	Geraniol	-874.511	H-bond Steric Electrostatic	Glu553 Gly441 and Ala478 None
1XK9	β-Citronellal	-866.123	H-bond Steric Electrostatic	Tyr481 Tyr470, Ala472 and Gly441 None
1XK9	P34 inhibitor	-123.588	H-bond Steric Electrostatic	2(Gly441) Gly441, Gln485, His440, Ala478 and Tyr470 None

Regarding LpxC protein (Table 3), the ciprofloxacin control had the lowest interaction energy and performed 2 hydrogen bonds with the residue Thr190. It is noted that this residue and Leu 200 participated in steric interactions with ciprofloxacin, germacrene D and germacrene D-4-ol.

Table 3. Energy values and types of interactions between poses and amino acid residues of LpxC protein.

PDB ID	*Pose*	Energy [kcal.mol^{-1}]	Interactions	
			Types	Residues
5VWM	Ciprofloxacin	-955.973	H-bond Steric Electrostatic	2(Thr190) 2(Leu18), Thr190, 3(Leu200), Val216, Gly209, 3(Ala214) and Asn213 None
5VWM	Germacrene D	-772.392	H-bond Steric Electrostatic	None 2(Leu200), 2(Phe193), Leu18, Phe191 and 2(Thr190) None
5VWM	Germacrene D-4-ol	-737.488	H-bond Steric Electrostatic	None Ala206, 2(Gly192), Phe191, 3(Thr190), Met62 and 2(Leu200) None

5VWM	Citronellyl acetate	-718.769	H-bond Steric Electrostatic	Thr190 2(Thr190), Met62 and Phe191 None
5VWM	C90 inhibitor	-141.354	H-bond Steric Electrostatic	2(Thr190), Phe191, 2(His264), Glu77, Asp241 and Lys 238 Ser210, 2(Thr190), Asp241, Glu77 and 2(His264) None

For PBP3 (Table 4), ciprofloxacin presented the lowest interaction energy, being observed 2 hydrogen bonds with Tyr407 and Tyr328. Germacrene D-4-ol had the second lowest interaction energy and made hydrogen bonds with residues Tyr409 and Thr487. This latter residue also participates in steric interactions with ciprofloxacin, germacrene D-4-ol and the JPP inhibitor. It is noteworthy that the residue Tyr409 made different interactions with this inhibitor and with all 4 poses that presented the best results.

Table 4. Energy values and types of interactions between poses and amino acid residues of PBP3.

PDB ID	*Pose*	Energy [kcal.mol^{-1}]	Interactions	
			Types	Residues
4KQO	Ciprofloxacin	-982.283	H-bond Steric Electrostatic	2(Tyr407) and 2(Tyr328) Tyr328, Thr404, Tyr498, 2(Tyr409), Thr487, 3(Arg489) and Tyr407 None
4KQO	Germacrene D-4-ol	-900.617	H-bond Steric Electrostatic	Tyr409 and Thr487 Ser294, 2(Asn351), 3(Thr487), Val333 and Ser349 None
4KQO	Germacrene D	-828.819	H-bond Steric Electrostatic	None Tyr328, 2(Tyr409) and Tyr498 None
4KQO	Citronellyl acetate	-775.039	H-bond Steric Electrostatic	Tyr409 and Arg489 Tyr409 None
4KQO	JPP inhibitor	-166.411	H-bond Steric Electrostatic	Ser485, Tyr328, 3(Tyr409), Arg489, Asn351 and 4(Thr487) 2(Ser485), Tyr409, Ala488, 4(Ser294) and 2(Thr487) Lys484

By analyzing Tables 2, 3 and 4, it can be observed that citronellyl acetate is among the study compounds with the best results for each protein, showing excellent results for demonstrating low interaction energies with all 3 proteins. In Figure 1 below, the types of interactions of some molecules with the proteins can be visualized.

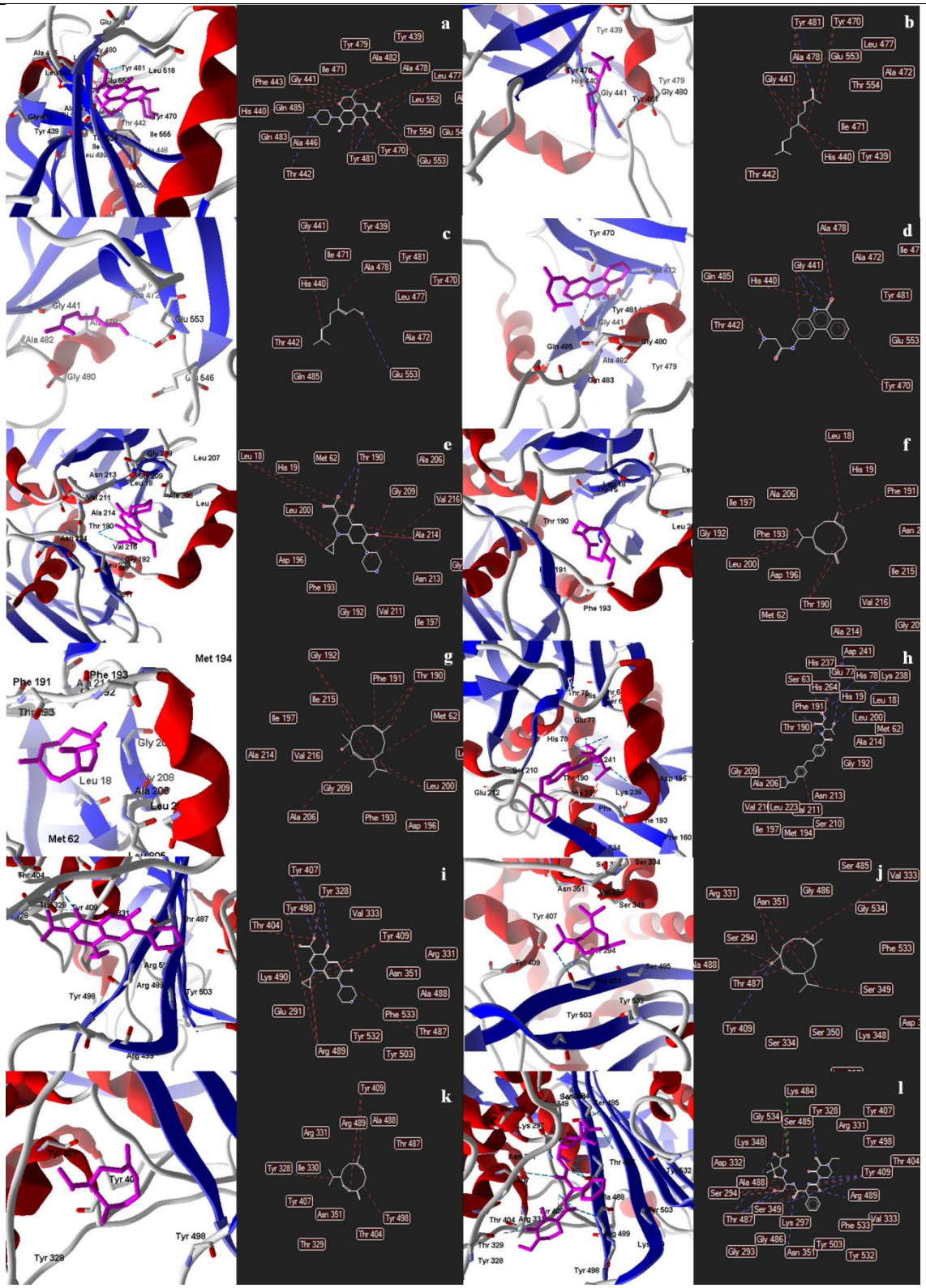


Figure 1. Interactions between poses and proteins. a) Levofloxacin with ExoA, b) Citronellyl acetate with ExoA, c) Geraniol with ExoA, d) P34 inhibitor with ExoA, e) Ciprofloxacin with LpxC, f) Germacrene D with LpxC, g) Germacrene D-4-ol with LpxC, h) C90 inhibitor with LpxC, i) Ciprofloxacin with PBP3, j) Germacrene D-4-ol with PBP3, k) Germacrene D with PBP3, l) JPP inhibitor with PBP3.

Conclusions

Based on molecular docking data, citronella essential oil compounds are promising against selected *P. aeruginosa* targets. Thus, it is important to obtain more data on these molecules by conducting different studies, such as prediction of biological activity, research on cytotoxicity risks and biological tests.

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