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Research Article

In silico disrupting quorum sensing of *porphyromonas gingivalis* via essential oils and coumarin derivatives

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Abstract

The emergence of *porphyromonas gingivalis* biofilm is a hallmark of risky burden diseases including Alzheimer's disease and atherosclerosis. The current study aims to screen some natural essential oil compounds and coumarin derivatives to interfere with quorum sensing of the bacterium and thus biofilm formation. A total of 20 ligands (10 essential oil molecules and 10 coumarin derivatives) were docked to *P.gingivalis* heme-binding protein HmuY using UCSF Chimera built-in AutoDock interface. Alongside, ADMET properties were also predicted via ADMETsar 2.0 and ProTox-II webservers. All of the selected ligands had higher free energy values than the reference inhibitor MES and native coumarin as well. Moreover, ADME parameters are in good agreement with Lipinski's rule of five. Nevertheless, the best molecules with top binding energy exhibited slight immunogenicity as well as carcinogenicity issues requiring *in vitro* confirmation. In conclusion, the tested ligands had better efficacy against *P.gingivalis* quorum sensing and biofilm.

More Information

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Introduction

Numerous microbiota commensal lives in harmony within mankind's oral cavity without harming the host tissues. These bacterial communities are composed of > 700 species forming a complex, structured biofilm attached to the tongue surface or subgingival region and embedded in a thick substance produced by those bacteria. This biofilm is a heterogeneous material comprised mainly of polysaccharides and DNA [1]. If the pathogenic bacteria constitute a major part, this puts a high risk to human health since, in the biofilm form, the pathogenic bacteria become more aggressive and resistant to host immune weapons and antimicrobials administered orally [2].

One of the most commonly found bacteria within the oral cavity is *Porphyromonas gingivalis* in addition to *Tannerella forsythia* and *Treponema denticola* (forming triad red complex) [3]. This Gram-negative, anaerobic joins the subgingival biofilm later [4]. This oral pathogen was at first thought to be associated with chronic periodontitis only. Indeed, nowadays it is well-known that *P.gingivalis* is linked to seemingly unpredictable types of diseases such as Alzheimer's disease (AD) [5], atherosclerosis [6], and cancers of the digestive canal [7]. This pathogenicity is mainly attributed to the high virulence pattern reflected by diverse virulence factors the bacterium uses to invade and colonize the underlying tissue plus the evading as well as interference with the immune cells' strategies [8].

Once the biofilm has been established, conventional antibiotics lack the usual effectiveness due to the polysaccharide barrier formed by the biofilm microbial community which hampers its accessibility. This prompted the creation of novel ways to deal with the pathogenic biofilm [9]. One such strategy is to target the quorum sensing (QS) pathway utilized by the bacterial community to sense the density of the species population and thus initiate biofilm formation and pathogenesis [10]. Thus, disrupting cell-cell communication eliminates unnecessary antibiotic usage and easily controls bacterial pathogenesis. This can be achieved through the use of some natural products which have proven their efficacy as antibiofilm as well as interference of the QS mechanisms [11]. Utilizing this concept, He, et al. [12] have

successfully used coumarin to interfere with the QS pathway of *P.gingivalis in silico* and *in vitro*.

This prompted us to screen some essential oil compounds and coumarin derivatives to control the pathogenicity of *P.gingivalis* toward periodontitis and other associated chronic diseases by targeting a heme-binding protein HmuY.

Materials and methods

Protein preparation

The crystal structure of the heme-free heme-binding protein of *P.gingivalis* HmuY complexed with 2-(n-morpholino)-ethane sulfonic acid (MES) (PDB ID: 6EWM) was retrieved from the protein data bank (PDB) as a PDB file. The crystal structure has a resolution of 1.4 Å. The protein is homodimer having 191 amino acid residues in total [13] Figure 1.

For preparing the receptor, all the heteroatoms, water, and ions were removed to obtain the native receptor protein. In addition, polar hydrogens were added and the charges were assigned.

Ligands preparation

A series of 10 common essential oil molecules and 10 coumarin derivatives were downloaded from PubChem and Chemspider datasets as SDF files and then converted to PDB format using Open Babel software [14]. After, the downloaded ligands were energy-minimized. Table 1 enumerates the tested ligands along with their structure. The selection criteria of ligands were on the observation that essential oils demonstrated their efficacy against QS of certain bacteria [15-17]. On the other hand, coumarin inhibited the QS of *P.gingivalis in silico* and *in vitro* [12].

Molecular docking

Protein and ligand preparation, energy minimization as well as molecular docking were performed using UCSF Chimera software (version 1.16) and its built-in autodock vina interface [18,19]. We performed a blind docking with a grid box covering all the receptor structures with dimensions 50 × 43 × 72 Å and centered at -2.355, 6.734 and 24.335 of X, Y and Z coordinates.

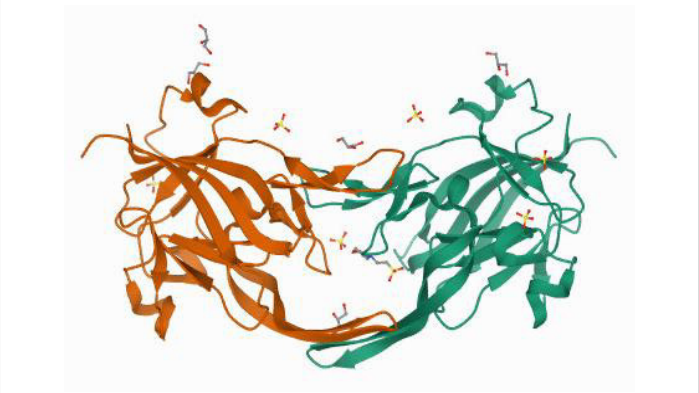


Figure 1: 3D structure of the heme-binding protein HmuY of P.gingivalis complexed with MES and sulfate ions (PDB ID: 6EWM).

Data visualization

To explore the docking interaction of best confirmation of the ligands with the highest binding affinities of both essential oils and coumarin derivatives, PyMOL [20], (surface mode) and Proteinplus webserver (2D diagram) [21], were employed for visualizations.

ADMET prediction

In order to evaluate the pharmacokinetics as well as drug-likeness of the selected ligands, the ADMETsar server was utilized [22]. Moreover, the ProTox-II platform [23], was used to assess the toxicity profiles of only the best docking compound of each group.

Results and discussion

Instead of the application of antibiotics that have no impact against pathogenic bacterial biofilms, some natural products emerge as potent factors interfering with QS and thus biofilm formation [24]. *P.gingivalis* is one bacterium found in oral biofilms that are correlated with many burden disorders like AD [25]. Therefore, upon targeting the QS pathway and preventing biofilm formation, a huge number of inflicted populations will benefit especially if the cure is of natural origin.

Docking analysis

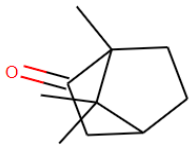
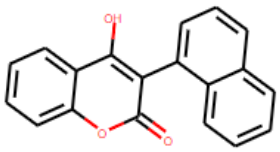
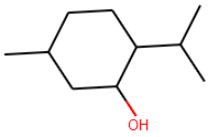
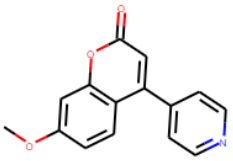
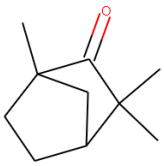
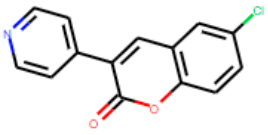
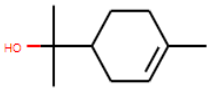
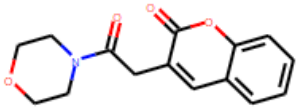
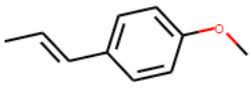
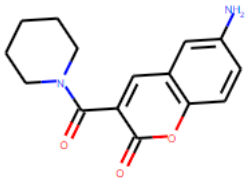
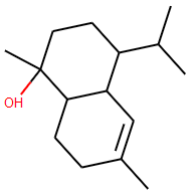
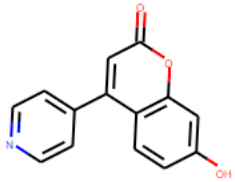
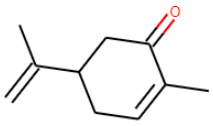
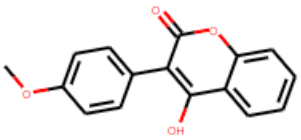
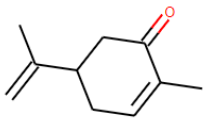
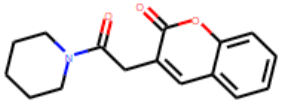
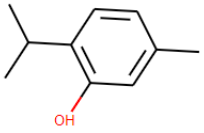
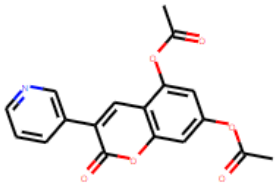
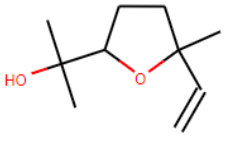
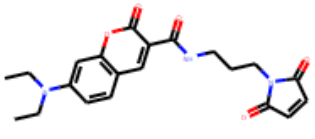
The binding affinities of the selected ligands were tested using the AutoDock vina interface within UCSF Chimera software. As shown in Table 2, all of the tested ligands belonging to essential oils exhibited higher binding affinity values compared to the reference inhibitor MES whose value was -5.5 kcal/mole. Cardinal gave maximum binding affinity of all essential oil molecules (-8.1 kcal/mole). The rest compounds' values were between -6.2 kcal/mole to -6.7 kcal/mole.

Conversely, coumarin and its derivatives had higher values of binding affinity than essential oils and the reference inhibitor MES (ranged from -8.5 kcal/mole to -10.4 kcal/mole). It should be noted that coumarin derivatives are better than coumarin moiety which gave an inhibition value of -7 kcal/mole.

It seems reasonable for coumarins to have higher free energy values than essential oils as they have at least two rings in their structure which make them more suitable to be fitted in the active center of the protein.

The top molecules with the highest binding energy of the two groups (cardinol and coumarin derivative 1) were analyzed further for their interaction with the active site of the target protein HmuY. Figure 2 illustrates the surface view of the receptor with the cardinol and coumarin derivative 1 bound to its active pocket as depicted using PyMOL software.

Table 1: List of the selected compounds to be docked against HmuY. The table lists the 2 groups (essential oils and coumarin derivatives) along with the corresponding structure.

Ligand	Structure	Ligand	Structure
Essential Oils		**Coumarin Derivatives**	
Camphor		Coumarin derivative 1 (6,7-Dimethoxy-4-(trifluoromethyl)coumarin)	
Menthol		Coumarin derivative 2 (Coumarin, 7-methoxy-4-(4-pyridyl)	
Fenchone		Coumarin derivative 3 (6-Chloro-3-(4-pyridyl)coumarin)	
α-Terpineol		Coumarin derivative 4 (3-((Morpholinocarbonyl)methyl)coumarin)	
Anethole		Coumarin derivative 5 (6-Amino-3-(piperidinocarbonyl)coumarin)	
Cardinol		Coumarin derivative 6 (6-Amino-3-(piperidinocarbonyl)coumarin)	
R-Carvone		Coumarin derivative 7 (7-Hydroxy-4-(4-Pyridyl)Coumarin)	
S-Carvone		Coumarin derivative 8 (3-(4-Methoxyphenyl)-4-Hydroxycoumarin)	
Thymol		Coumarin derivative 9 (3-((Piperidinocarbonyl)methyl)coumarin)	
Trans-linalool oxide		Coumarin derivative 10 (7-Diethylamino-3-[N-(3-maleimidopropyl)carbamoyl]coumarin)	

In addition, the 2D diagram of interaction is also shown in Figure 2 as predicted via proteins *plus* server.

Since coumarin derivative 1 has more energy values in comparison with the essential oil cardinol, it has more H-bonds and VdW interactions within the active site of the target protein HmuY. Coumarin derivative 1 has more interactions with Tyr 173 (via H-bonds) and Leu 162, Phe 156, and Ala 157 (through VdW interactions) while Asp 81, as well as Tyr 80, appear to have mutual interactions with both of them (Figure 2).

Prediction of ADMET parameters

It is necessary to predict and calculate the pharmacokinetic properties of the docked ligands to figure out their druggability. Table 3 lists the ADME properties of all ligands obtained from the ADMETsar 2.0 webserver.

As shown in Table 3, all of the tested ligands in the two groups are in a domain, i.e. they fit well to Lipinski's rule of five. This means the good druggability profile of the selected compounds. However, a toxicity profile must be carried out to evaluate the possible toxicity issues of the docked molecules to be lead candidates as inhibitors of the QS of *P.gingivalis*. Table 4 depicts the toxicity paramters of cardinol and coumarin derivative 1 (the best-docked molecules).

Concerning toxicity assessment, cardinol exhibited slight immunogenicity (probability 0.69) and showed activity toward MMP. Similarly, coumarin derivative 1 was found to have carcinogenicity (probability 0.55) and activity toward

Table 2: Molecular docking results of all selected compounds from the two groups against the target protein 6EWM.

Ligand	Binding affinity	Ligand	Binding affinity
Camphor	-6.5	Coumarin	-7
Menthol	-6.5	Derivative 1	-10.4
Fenchone	-6.4	Derivative 2	-8.5
Terpineol	-6.7	Derivative 3	-8.5
Anethole	-6.2	Derivative 4	-8.9
Cardinol	-8.1	Derivative 5	-9.8
R-Carvone	-6.4	Derivative 6	-8.7
S-Carvone	-6.6	Derivative 7	-8.7
Thymol	-6.6	Derivative 8	-9.8
Trans-linalool oxide	-6.2	Derivative 9	-8.9
MES	-5.5	Derivative 10	-9.2

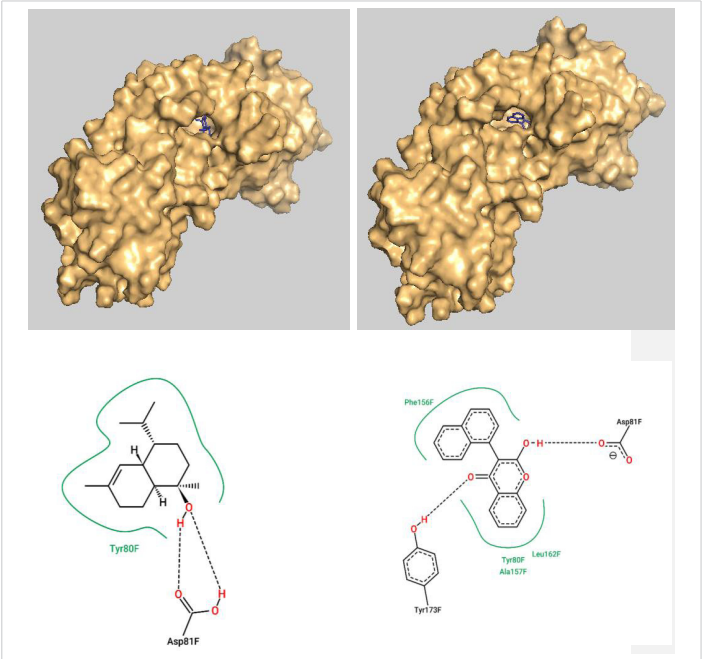


Figure 2: Surface view of the target protein with the cardinol (Top left) and coumarin derivative (Top right) bound to the active pocket. Also, the 2D diagram of cardinol interaction (bottom left) and coumarin derivative 1 (bottom right) with the active site residues.

Table 3: Obtained ADME properties of all selected ligands in the two groups along with their applicability domain.

Ligand	MW	AlogP	HA	HD	RB	Applicability Domain
Essential oils						
Camphor	152.24	2.4	1	0	0	In domain
Menthol	156.27	2.44	1	1	1	In domain
Fenchone	152.24	2.4	1	0	0	In domain
Terpineol	154.25	2.5	1	1	1	In domain
Anethole	148.2	2.73	1	0	2	In domain
Cardinol	222.37	3.78	1	1	1	In domain
R-Carvone	150.22	2.49	1	0	1	In domain
S-Carvone	150.22	2.49	1	0	1	In domain
Thymol	150.22	2.82	1	1	1	In domain
Trans-linalool oxide	170.25	1.88	2	1	2	In domain
MES	195.24	-0.79	4	1	3	In domain
Coumarin derivatives						
Coumarin	146.14	1.79	2	0	0	In domain
Derivative 1	288.3	4.32	3	1	1	In domain
Derivative 2	253.26	2.86	4	0	2	In domain
Derivative 3	257.68	3.51	3	0	1	In domain
Derivative 4	273.29	1.19	4	0	2	In domain
Derivative 5	272.3	2	4	1	1	In domain
Derivative 6	239.23	2.56	4	1	1	In domain
Derivative 7	268.27	3.17	4	1	2	In domain
Derivative 8	271.32	2.35	3	0	2	In domain
Derivative 9	339.3	2.71	7	0	3	In domain
Derivative 10	397.43	1.68	6	1	8	In domain

MW: Molecular Weight; HA: Hydrogen Acceptor; HD: Hydrogen Donor; RB: Rotatable Bonds.

Table 4: ProTox-II server results of toxicity profiles of cardinol and coumarin derivative 1.

Target	Cardinol		Coumarin derivative 1	
	Prediction	Probability	Prediction	Probability
Hepatotoxicity	Inactive	0.82	Inactive	0.67
Carcinogenicity	Inactive	0.66	Active	0.55
Immunotoxicity	Active	0.69	Inactive	0.81
Mutagenicity	Inactive	0.91	Inactive	0.83
Cytotoxicity	Inactive	0.87	Inactive	0.62
Aryl hydrocarbon Receptor (AhR)	Inactive	0.98	Active	0.55
Androgen Receptor (AR)	Inactive	0.87	Inactive	0.98
Aromatase	Inactive	0.92	Inactive	0.7
Estrogen Receptor Alpha (ER)	Inactive	0.8	Inactive	0.53
Estrogen Receptor Ligand Binding Domain (ER-LBD)	Inactive	0.83	Inactive	0.74
Peroxisome Proliferator-Activated Receptor Gamma (PPAR-Gamma)	Inactive	1	Inactive	0.59
Heat shock factor response element (HSE)	Inactive	0.69	Inactive	0.97
Mitochondrial Membrane Potential (MMP)	Active	0.5	Active	0.6
Phosphoprotein (Tumor Supressor) p53	Inactive	0.99	Inactive	0.75

AhR as well as MMP. In short, the predicted toxicity of cardinol is class IV whereas coumarin derivative 1 is class III as calculated by the ProTox-II platform. This work suggests the application of the mentioned ligands as a means to control the biofilm of *P.gingivalis* found within the oral cavity and its associated burden diseases such as dental plaques, Alzheimer's disease, and atherosclerosis. By approving their potency *in silico*, the best 2 ligands should find their way *in vitro* setting to confirm the computational prediction by wet lab experiments as coupled by [12] .

Conclusion

According to the data obtained in this study, we concluded the stable inhibition of *P.gingivalis* QS via some natural essential oil molecules (best of which was cardinol with a binding affinity of -8.1 kcal/mole) and some coumarin derivatives. The best of which was derivative 1 exhibiting a binding affinity of -10.4 kcal/mole. Furthermore, the docked ligands demonstrated good ADME properties which candidate them superiorly above the reference inhibitor MES and even the native coumarin. However, toxicity prediction results revealed that cardinol was immunogenic and coumarin derivative 1 was carcinogenic. Thereby, we recommend after confirmation of results *in vitro* experiments to assay the immunogenicity and carcinogenicity of the cardinol and coumarin derivative 1 as well or at least can be utilized as a lead pharmacophore through which safer pharmaceuticals can be designed and improved.

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