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Targeting the Bap1 protein of *Vibrio cholerae* for screening potent inhibitors and predicting the mutant protein stability: in silico analyses

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Abstract

Background: Bap1 is reported to be a major protein in *Vibrio cholerae* which aids in biofilm formation. Hence, inhibition of the protein molecule can no longer support the colonization of bacteria and induction of point mutations at specific amino acid residues tend to reduce the protein stabilization. **The aim of the study:** To target this Bap1 structural protein for screening potent inhibitors of ligands by means of *in silico* analyses. **Materials and methods:** A total of 30 compounds divided into three groups such as synthetic antimicrobial drugs, phytochemicals and marine compounds comprising of ten ligands in each group were tested against Bap1. In addition to this, mutations were induced at GLN: 518, HIS: 520 and ASN: 679 positions to determine the stability of the mutant Bap1 protein using bioinformatic tools. **Results:** Of the 30 docked compounds, doxycycline, ichangin and Ageloxime D exhibited the highest binding affinities of -8.5 kcal/mol, -9.3 kcal/mol and -8.8 kcal/mol respectively from the three groups. The ADME properties show the druglikeness of the test compounds to be used for treatment procedures. Protein-ligand interactions were visualized which infer that both doxycycline and ichangin form five conventional hydrogen bonds while Ageloxime D could form three hydrogen bonds with different amino acid residues of the protein. Further, the van der Waals' interactions are also found to be similar in number among doxycycline and ichangin whereas, it is less in case of Ageloxime D but, the π -interactions are high in this compound comparatively. The carbon-hydrogen bonding for these three compounds with the amino acid residues of the Bap1 protein have also been discussed. **Conclusion:** Thus, natural compounds can eventually replace the over use of synthetic drugs as antibiofilm agents. Also, inducing point mutations to this protein can potentially destabilize its structure.

Keywords: Bap1; in silico analyses; binding affinity; ichangin; antibiofilm agents; mutant protein stability

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Introduction. Bacterial biofilm is the colonization of one or more species of bacteria together onto a specific substratum. The adhesion of these bacterial cells is brought up by the extracellular polymeric substances that form matrices favoring high resistance to the embedded microorganisms against adverse conditions [1]. Most of the pathogenic bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus viridans* and *Vibrio cholerae* have been found to form biofilms within the host body and in the environment as well [2]. Also, it is quite difficult to treat bacterial infections that have formed biofilm previously.

Vibrio cholerae, a gram negative, comma shaped and motile bacterium has the tendency to easily colonize on marine bodies [3, 4]. It can cause a serious disease called 'cholera' in humans when contaminated water and food sources are ingested. Bap1 (Biofilm associated protein 1) protein is considered to be the major factor for biofilm formation in *V. cholerae* [5]. This protein is abundantly expressed during biofilm formation, enabling adhesion of bacterial cells to the host surface. It has also been reported that the Bap1 protein plays a structural rather than functional role in *V. cholerae* [5]. The continual use of antibiotics to treat infections may lead to multi drug resistance in bacteria. Hence, there is a need for screening antibiofilm agents that can inhibit further development of biofilm which are naturally available in plants and marine organisms in addition to the synthetic antimicrobial drugs.

The implementation of computational procedures is becoming widely adopted by the researchers for drug discovery, analyzing the protein structures and predicting the stability of mutant proteins from that of the respective wild types [6, 7]. Here, the authors have performed molecular docking techniques in order to screen potential compounds that inhibited the Bap1 protein of *V. cholerae* as a measure of controlling biofilm production by the bacterium. The docked compounds were also evaluated for druglikeness to treat the disease by reducing the risk of biofilm formation in humans. Along with this, Bap1 protein structure was mutated at several positions that interact

more with the test ligand molecules and the mutant protein stability was calculated.

The aim of the study. To target this Bap1 structural protein for screening potent inhibitors of ligands by means of *in silico* analyses.

Materials and Methods

Protein preparation

The structure of the protein was retrieved from the RCSB-PDB (The Research Collaboratory for Structural Bioinformatics- Protein Data Bank) database. The Bap1 protein with PDB Id: 6MLT bearing the title "Crystal structure of the *V. cholerae* biofilm matrix protein Bap1" was used as the protein of interest in this study [8]. The protein molecule was retrieved in the '.pdb' format. All the non-standard amino acid residues and water molecules were removed from the Bap1 protein structure and saved the file in the same format using the Chimera software.

Ligand preparation

A total of 30 compounds grouped into three different groups namely synthetic antimicrobial drugs, phytochemicals [9] and marine compounds which are listed in Table 1 were tested for the antibiofilm activity of *V. cholerae* bacterium by interacting with the Bap1 protein. The three-dimensional structures of the ligands were retrieved from ChemSpider and PubChem databases in '.mol' and '.sdf' formats respectively. The ligands were also screened for druggability based on Lipinski rule of five simultaneously.

Molecular docking

The retrieved synthetic antimicrobial drugs, phytochemicals and marine compounds were docked against the Bap1 protein structure. The molecular docking was carried out using Chimera software [10] through the AutoDock vina software [11]. The grid parameters were set as $x=15.0465$, $y=30.9405$ and $z=115.82$ and the size $x=65.3508$, $y=58.319$ and $z=80.6473$ for the interaction to be checked. The AutoDock tool provides the results in kcal/mol based on hydrogen bonding, electrostatic energies, desolvation and van der Waals force [10]. The binding poses with maximum binding affinities and lowest binding energies were chosen for all the compounds.

Table 1

List of ligand molecules

Group	Name of the compound	Molecular formula	Molecular weight (Da)	Druggability based on Lipinski's rules
Synthetic antimicrobial drugs	Amoxicillin	C ₁₆ H ₁₉ N ₃ O ₅ S	365.404	Yes
	Chloramphenicol	C ₁₁ H ₁₂ Cl ₂ N ₂ O ₅	323.129	Yes
	Ciprofloxacin	C ₁₇ H ₁₈ FN ₃ O ₃	331.341	Yes
	Doxycycline	C ₂₂ H ₂₄ N ₂ O ₈	444.435	Yes
	Metronidazole	C ₆ H ₉ N ₃ O ₃	171.154	Yes
	Norfloxacin	C ₁₆ H ₁₈ FN ₃ O ₃	319.331	Yes
	Ofloxacin	C ₁₈ H ₂₀ FN ₃ O ₄	361.367	Yes
	Sulfacetamide	C ₈ H ₁₀ N ₂ O ₃ S	214.242	Yes
	Sulfathiazole	C ₉ H ₉ N ₃ O ₂ S ₂	255.317	Yes
Sulphamethoxazole	C ₁₀ H ₁₁ N ₃ O ₃ S	253.278	Yes	
Phytochemicals	Ajoene	C ₉ H ₁₄ OS ₃	234.4019	Yes
	Carvacrol	C ₁₀ H ₁₄ O	150.218	Yes
	Chelerythrine	C ₂₁ H ₁₈ NO ₄	348.371	Yes
	Curcumin	C ₁₂ H ₂₀ O ₆	368.3799	Yes
	Emodin	C ₁₅ H ₁₀ O ₅	270.237	Yes
	Hordenine	C ₁₀ H ₁₅ NO	165.232	Yes
	Ichanganin	C ₂₆ H ₃₂ O ₉	488.527	Yes
	Polygodial	C ₁₅ H ₂₂ O ₂	234.334	Yes
	Warburganal	C ₁₅ H ₂₂ O ₃	250.333	Yes
Zingerone	C ₁₁ H ₁₄ O ₃	194.227	Yes	
Marine compounds	Ageloxime D	C ₂₆ H ₄₀ N ₅ O ⁺	438.6	Yes
	Butenolide	C ₄ H ₄ O ₂	84.073	Yes
	Clathrocin	C ₁₁ H ₁₃ N ₅ O	231.254	Yes
	Hymenidin	C ₁₁ H ₁₂ BrN ₅ O	310.150	Yes
	Manoalide	C ₂₅ H ₃₆ O ₅	416.550	Yes
	Motualevic acid	C ₁₆ H ₂₃ Br ₂ NO ₂	439.2	Yes
	Nakijiquinone G	C ₂₆ H ₃₅ N ₃ O ₃	437.574	Yes
	Nigrosporapyrone D	C ₈ H ₈ O ₄	168.15	Yes
	Oroidin	C ₁₁ H ₁₁ Br ₂ N ₅ O	389.046	Yes
Secomanoalide	C ₂₅ H ₃₆ O ₅	416.550	Yes	

ADME calculation

The Absorption, Distribution, Metabolism and Excretion (ADME) properties for the selected compounds were calculated using SwissADME [12] online server by uploading the canonical SMILES (Simplified Molecular Input Line Entry System) format of the top five ligand molecules from each group (synthetic antimicrobial drugs, phytochemicals and marine compounds). The ADME properties define the drug-likeness of the chosen ligand compounds.

Analysis of protein-ligand interactions

Similarly, the ligand molecules interacting with the amino acid residues were determined for the topmost compound showing highest binding affinity from each group by means of Biovia Discovery Studio Visualiser.

The hydrogen bonding, hydrophobic interaction, π - interactions and covalent bonds can be visualized between the interacting molecules of the protein.

Protein stability prediction

The stability of the Bap1 protein was predicted by substituting the amino acid residues which frequently took part in forming hydrogen bonds among the best ligand compounds using CUPSAT server [13]. The amino acids were mutated with nineteen other amino acids in the respective positions and the mutant stability was estimated.

Results and Discussion

Molecular docking

The results for molecular docking of all the synthetic antimicrobial drugs, phytochemi-

cals and marine compounds with the Bap1 protein are tabulated in Table 2. In synthetic antimicrobial drugs group, the drug doxycycline shows high binding affinity of -8.5 kcal/mol and the drug with least affinity is metronidazole with an affinity of -5.1 kcal/mol. In the phytochemicals group, ichangin has the maximum binding affinity of about -9.3 kcal/mol and the phytochemical with least affinity was found to be ajoene (-4.1 kcal/mol). Similarly, the compound ageloxime D shows high affinity of -8.8 kcal/mol and butenolide has the minimum binding affinity of about -4.0 kcal/mol in the marine compounds group. Comparatively, natural compounds show greater affinity towards the antibiofilm capability than synthetic chemicals. The drug, doxycycline has shown binding affinity of about -9.8 kcal/mol against ACE2 receptor to prevent the viral entry into the host cells [14]. Amoxicillin showed

binding energy of about -7.0 kcal/mol towards *Staphylococcus aureus* Pyruvate Kinase targeted as a measure of antimicrobial activity [15]. Curcumin binds to the *S. aureus* biofilm forming protein with an affinity of -6.33 kcal/mol [16]. The marine metabolite hymenidin has shown inhibition of the SARS- CoV2 protease with a binding energy of -6.4 kcal/mol [17]. Likewise, a limonoid phytochemical, ichangin could bind with the same COVID-19 protease exhibiting a binding affinity of about -8.4 kcal/mol docked as a measure of repurposing the plant chemical as antiviral agent [18]. Natural compounds are more capable of inhibiting the biofilm matrix protein due to their efficient quorum sensing (QS) blocking mechanism comparatively higher than synthetic constituents [19]. Further, natural compounds are the best treatment options for bacteria that have developed antibiotic resistance [20].

Table 2

Binding affinity values of ligands with Bap1 received in AutoDock vina

Group	Name of the compound	Binding affinity (kcal/mol)
Synthetic antimicrobial drugs	Amoxicillin	-7.2
	Chloramphenicol	-6.4
	Ciprofloxacin	-7.9
	Doxycycline	-8.5
	Metronidazole	-5.1
	Norfloxacin	-7.3
	Ofloxacin	-7.6
	Sulfacetamide	-6.0
	Sulfathiazole	-6.5
Sulphamethoxazole	-6.8	
Phytochemicals	Ajoene	-4.1
	Carvacrol	-6.0
	Chelerythrine	-7.8
	Curcumin	-6.7
	Emodin	-7.8
	Hordenine	-5.6
	Ichangin	-9.3
	Polygodial	-6.5
	Warburganal	-6.5
Zingerone	-5.9	
Marine compounds	Ageloxime D	-8.8
	Butenolide	-4.0
	Clathrocin	-6.2
	Hymenidin	-6.4
	Manoalide	-7.5
	Motualevic acid	-5.0
	Nakijiquinone G	-8.0
	Nigrosporapyrone D	-6.1
	Oroidin	-6.1
Secomanoalide	-6.8	

ADME calculation

The ADME calculation shows that all the top five compounds with maximum binding affinities from each group tend to exhibit drug-likeness with no or less violations for a few compounds. The results are given in Table 3. It can be suggested that almost all the test compounds screened for ADME estimation can be considered for preclinical trials except chelerythrine and polygodial which tend to cross the brain-blood barrier. Both doxycycline and amoxicillin are generally less absorbed in the gastrointestinal tract and hence, the route of administration shall be changed during treatment procedures. The marine compounds screened for ADME estimation did show less solubility in water except hymenidin as predicted by the SwissADME online tool. It has been shown that ichangin can inhibit the biofilms in *E. coli* at an Inhibitory Concentration (IC₂₅) of 28.3 μM [21]. Ichangin can be used against SARS- CoV2 virus after approving it's

ADME properties [22]. Manoalide exerted anti-inflammatory effects and hence can be administered as a drug considering its druglikeness [23]. Ageloxime D was able to possibly inhibit *Cryptococcus neoformans* with IC₅₀ value of nearly 5.94 μg/mL proving its ability to act as a drug against bacteria [24]. The natural compounds may be administered in combinations with several antimicrobials which can increase the interactions with their specific targets inside the pathogens [25]. These combinations can reduce the toxicity and minimum inhibitory concentrations of many antibiotics as well to treat severe bacterial infections [26]. The previously developed fatal bacterial biofilms will not get easily vanished since, bacteria are protected within the matrix. In such cases, synergistic combinations of natural antibiofilm compounds can inhibit biofilms therefore, exposing the embedded bacteria to the synthetic antimicrobials that contain bactericidal activities and get killed [27].

Table 3

ADME determination for the top five compounds with high binding affinity from each group

Group	Compound	Water solubility	GI absorption	Brain-blood barrier permeant	Skin permeation (-Log K _p)	Bioavailability score
Synthetic antimicrobial drugs	Doxycycline	Soluble	Low	No	-8.63 cm/s	0.11
	Ciprofloxacin	Soluble	High	No	-9.09 cm/s	0.55
	Ofloxacin	Soluble	High	No	-8.78 cm/s	0.55
	Norfloxacin	Soluble	High	No	-8.98 cm/s	0.55
	Amoxicillin	Soluble	Low	No	-9.94 cm/s	0.55
Phyto-chemicals	Ichangin	Soluble	High	No	-8.73 cm/s	0.55
	Chelerythrine	Moderately soluble	High	Yes	-5.17 cm/s	0.55
	Emodin	Soluble	High	No	-6.02 cm/s	0.55
	Curcumin	Moderately soluble	High	No	-6.28 cm/s	0.55
	Polygodial	Soluble	High	Yes	-5.44 cm/s	0.55
Marine compounds	Ageloxime	Poorly soluble	High	No	-4.26 cm/s	0.55
	Nakijiquinone G	Poorly soluble	High	No	-5.89 cm/s	0.55
	Manoalide	Moderately soluble	High	No	-6.32 cm/s	0.55
	Secomanoalide	Moderately soluble	High	No	-5.93 cm/s	0.55
	Hymenidin	Soluble	High	No	-7.50 cm/s	0.55

Protein-ligand interactions

The interaction between the Bap1 matrix protein of *Vibrio cholerae* and the compounds-

doxycycline, ichangin and ageloxime D are depicted in Figures 1, 2 and 3 respectively. Doxycycline forms five conventional hydrogen bonds with GLN:518, HIS: 520, THR:

677, ASN: 679 and LEU: 690 residues of the protein. In addition to this, ASN: 249, SER: 302, SER: 310, ALA: 311, ASN: 336 ASP: 517, TRP: 675 and HIS: 678 promote van der Waals interactions with this compound. Similar to this interaction, the phytochemical ichangin also forms five conventional hydrogen bonds with ASN: 249, THR: 312, GLN: 518, HIS: 520 and ASN: 679 residues. SER: 302, SER: 310, ASP: 517, TRP: 675, LEU: 676, THR: 677, PRO: 689 and LEU: 690 support van der Waals interaction. Along with this, ichangin forms two carbon-hydrogen bonds with ALA: 311 and HIS: 678 and one π -alkyl interaction with PRO: 307 amino acid. Likewise, the marine compound ageloxime D,

exhibiting the highest affinity in the group, comparatively forms only three conventional hydrogen bonds (one with HIS: 678 and two bonds with ARG: 687). The van der Waals' attractions are also less with this compound. But the π -interactions are remarkably more in ageloxime D involving ARG: 649, PHE: 658, ALA: 663, ALA: 671, ILE: 686 and PRO: 689 (totally six interactions) amino acid residues of the Bap1 protein. In a literature, it is reported that doxycycline was able to form six hydrogen bonds with the main protease of COVID-19 [28]. Ichangin being a limonoid could interact with HIS: 41, GLU: 166 and GLN: 189 residues of the same protein forming three hydrogen bonds [18].

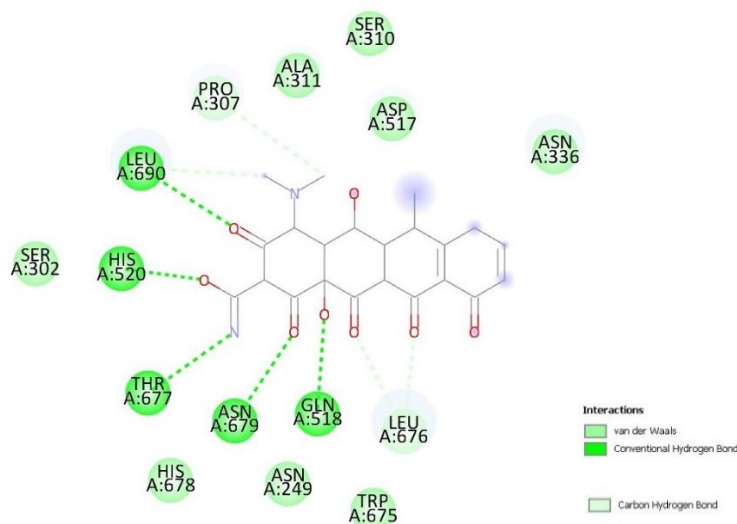


Fig. 1. Interaction of doxycycline with the amino acids of Bap1 protein

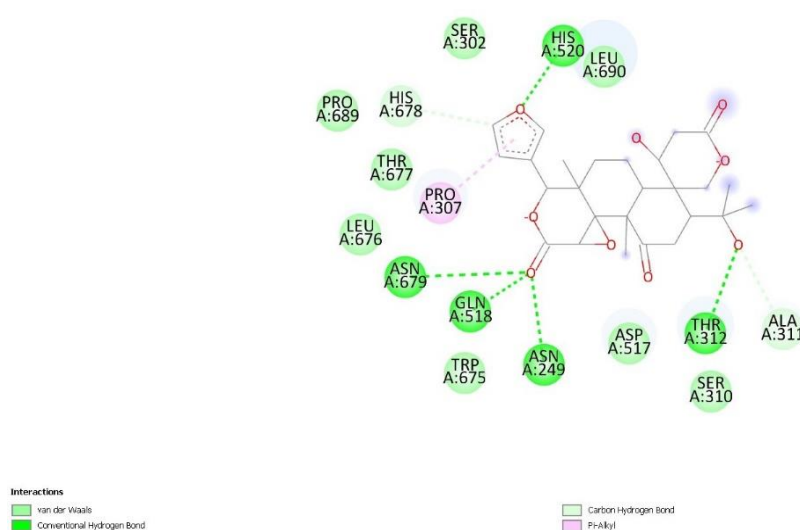


Fig. 2. Interaction of ichangin with the amino acids of Bap1 protein

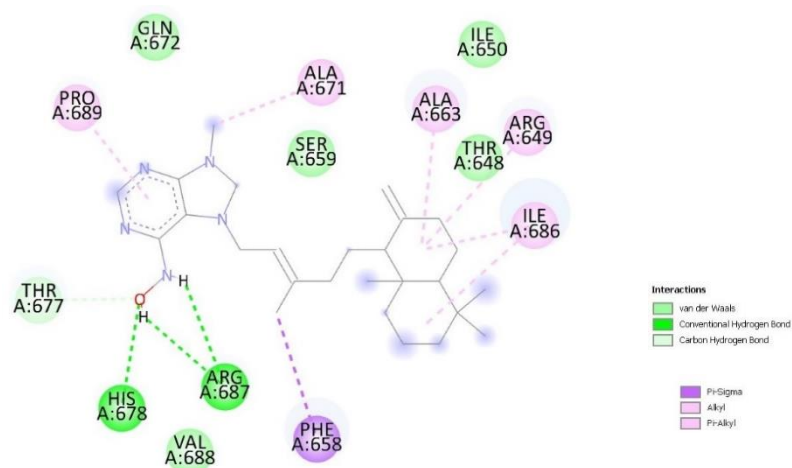


Fig. 3. Interaction of Ageloxime D with the amino acids of Bap1 protein

Protein stability prediction

The amino acids that frequently took part in conventional hydrogen bond formation among doxycycline, ichangin and ageloxime D were found to be GLN: 518, HIS: 520 and ASN: 679. Hence, these three amino acids were substituted with nineteen other amino acid residues at the positions 518, 520 and 679 to determine the mutant protein stability which is tabulated in Table 4. Based on the protein stability prediction of Bap1 protein, it can be inferred that destabilization of the protein structure can occur if glutamic acid ($\Delta\Delta G = -0.39$ kcal/mol) gets substituted at the 518th position. Similarly, valine, leucine and

isoleucine are the only three residues which are stabilizing amino acids at the 520th position. The destabilizing residues at the 679th position are glycine, proline, tryptophan, threonine, glutamine, tyrosine, cysteine and aspartic acid when ASN: 679 gets substituted due to mutations according to the predictions. It is reported that nearly, ten residues favor instability at the desired positions of mutant CYP3A4 protein using the same server as used in this study [29]. The non-synonymous mutation in the helicase was determined to be $\Delta\Delta G = 0.377$ kcal/mol by shifting from histidine to tyrosine (H39Y) [30].

Table 4

Predicted $\Delta\Delta G$ values for the substituted amino acids at 518, 520 and 679 positions

Substituted amino acids at GLN: 518	$\Delta\Delta G$ (kcal/mol)	Substituted amino acids at HIS: 520	$\Delta\Delta G$ (kcal/mol)	Substituted amino acids at ASN: 679	$\Delta\Delta G$ (kcal/mol)
GLY	7.08	GLY	-3.2	GLY	-1.84
ALA	5.26	ALA	-1.71	ALA	0.72
VAL	4.9	VAL	2.17	VAL	2.26
LEU	5.95	LEU	3.69	LEU	1.27
ILE	5.64	ILE	3.02	ILE	0.06
MET	8.6	MET	-0.33	MET	3.08
PRO	3.48	PRO	-0.37	PRO	-1.88
TRP	3.16	TRP	-4.4	TRP	-1.69
SER	3.74	SER	-3.77	SER	0.44
THR	4.32	THR	-2.01	THR	-0.48
PHE	9.3	PHE	-0.36	PHE	3.16
LYS	10.35	GLN	-6.84	GLN	-0.08
TYR	3.03	LYS	-0.32	LYS	4.42
ASN	4.64	TYR	-1.91	TYR	-2.19
CYS	2.27	ASN	-5.76	CYS	-2.51
GLU	-0.39	CYS	-2.16	GLU	1.41
ASP	4.74	GLU	-3.32	ASP	-2.44
ARG	6.01	ASP	-5.08	ARG	2.83
HIS	1.77	ARG	-1.75	HIS	0.92

Conclusion. The *in silico* results suggest that targeting the major biofilm forming protein, Bap1 in *Vibrio cholerae* can potentially inhibit the production of biofilm among the bacterium. Other than synthetic antimicrobial drugs, phytochemicals and marine compounds can also be used as antibiofilm agents [16]. Molecular docking, ADME calculation and protein-ligand interaction analyses of the compounds exhibiting highest binding affinity from each group of ligands used in this study infer that doxycycline, ichangin and Ageloxime D are potent inhibitors of the Bap1 protein. On the whole, natural compounds are capable of inhibiting the biofilm matrix protein than synthetic constituents. These compounds may be administered in combinations with the antimicrobials in order to treat severe infections of the bacterium which may be concluded by performing preclinical experiments before the trials. Also, the use of ichangin and Ageloxime D can work better in the environment as well by preventing the colonization of *V. cholerae* on land and water bodies too [9]. Thus, screening, production, isolation and purification of natural drugs from plant and marine organisms will have promising effects in the treatment and early management of diseases [31]. Further, the mutant Bap1 protein stability was assessed by inducing mutations at the most interactive amino acid positions. The Bap1 mutants are found to have reduced structural stabilizations which may show deformities during biofilm formation. It could also lead to malformations in the attachment of bacterial colonies onto the desired substrata so that the mutant *V. cholerae* may not get protected from adverse conditions allowing the bacteria to be easily killed when exposed to physical or chemical agents [27].

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Conflict of interests

The authors have no conflict of interest to declare.

References

1. Yin W, Wang Y, Liu L, et al. Biofilms: The Microbial “Protective Clothing” in Extreme

Environments. *International Journal of Molecular Sciences*. 2019;20(14):3423. DOI: <https://doi.org/10.3390/ijms20143423>

2. Khatoon Z, McTiernan CD, Suuronen EJ, et al. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. *Heliyon*. 2018;4(12):1067. DOI: <https://doi.org/10.1016/j.heliyon.2018.e01067>

3. Reidl J, Klose KE. *Vibrio cholerae* and cholera: out of the water and into the host. *FEMS Microbiology Reviews*. 2002;26(2):125-139. DOI: <https://doi.org/10.1111/j.1574-6976.2002.tb00605.x>

4. Lekshmi N, Joseph I, Ramamurthy T, et al. Changing facades of *vibrio cholerae*: An enigma in the epidemiology of cholera. *Indian Journal of Medical Research*. 2018;147(2):133-141. DOI: https://doi.org/10.4103/ijmr.IJMR_280_17

5. Duperthuy M, Sjöström AE, Sabharwal D, et al. Role of the *Vibrio cholerae* Matrix Protein Bap1 in Cross-Resistance to Antimicrobial Peptides. *PLoS Pathogens*. 2013;9(10):e1003620. DOI: <https://doi.org/10.1371/journal.ppat.1003620>

6. Katsila T, Spyroulias GA, Patrinos GP, et al. Computational approaches in target identification and drug discovery. *Computational and Structural Biotechnology Journal*. 2016;14:177-184. DOI: <https://doi.org/10.1016/j.csbj.2016.04.004>

7. Sanavia T, Birolo G, Montanucci L, et al. Limitations and challenges in protein stability prediction upon genome variations: towards future applications in precision medicine. *Computational and Structural Biotechnology Journal*. 2020;18:1968-1979. DOI: <https://doi.org/10.1016/j.csbj.2020.07.011>

8. Kaus K, Biester A, Chupp E, et al. The 1.9Å crystal structure of the extracellular matrix protein Bap1 from *Vibrio cholerae* provides insights into bacterial biofilm adhesion. *Journal of Biological Chemistry*. 2019;294(40):14499-14511. DOI: <https://doi.org/10.1074/jbc.RA119.008335>

9. Mishra R, Panda AK, de Mandal S, et al. Natural Anti-biofilm Agents: Strategies to Control Biofilm-Forming Pathogens. *Frontiers in Microbiology*. 2020;11:2640. DOI: <https://doi.org/10.3389/fmicb.2020.566325>

10. Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera—A visualization system for exploratory research and analysis. *Journal of Computational Chemistry*. 2004;25(13):1605-1612. DOI: <https://doi.org/10.1002/jcc.20084>

11. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and

- multithreading. *Journal of Computational Chemistry*. 2010;31(2):455-461. DOI: <https://doi.org/10.1002/jcc.21334>
12. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*. 2017;7(1):42717. DOI: <https://doi.org/10.1038/srep42717>
13. Parthiban V, Gromiha MM, Schomburg D. CUPSAT: prediction of protein stability upon point mutations. *Nucleic Acids Research*. 2006;34(2):W239-W242. DOI: <https://doi.org/10.1093/nar/gkl190>
14. Kabir ER, Mustafa N, Nausheen N, et al. Exploring existing drugs: proposing potential compounds in the treatment of COVID-19. *Heliyon*. 2021;7(2):e06284. DOI: <https://doi.org/10.1016/j.heliyon.2021.e06284>
15. Degfie T, Endale M, Tafese T, et al. In vitro antibacterial, antioxidant activities, molecular docking, and ADMET analysis of phytochemicals from roots of *Hydnora johannis*. *Applied Biological Chemistry*. 2022;65:76. DOI: <https://doi.org/10.1186/s13765-022-00740-8>
16. Lahiri D, Nag M, Dey S, et al. Phytochemicals of *Curcuma longa* extract are more effective against bacterial biofilm than pure curcumin only: An in-vitro and in-silico analysis. *Kuwait Journal of Science*. 2021;48(2):8310. DOI: <https://doi.org/10.48129/kjs.v48i2.8310>
17. Murugesan S, Ragavendran C, Ali A, et al. Screening and Druggability Analysis of Marine Active Metabolites against SARS-CoV-2: An Integrative Computational Approach. *Journal of Translational Medicine*. 2023;3(1):27-41. DOI: <https://doi.org/10.3390/ijtm3010003>
18. Giofrè SV, Napoli E, Iraci N, et al. Interaction of selected terpenoids with two SARS-CoV-2 key therapeutic targets: An in silico study through molecular docking and dynamics simulations. *Computers in Biology and Medicine*. 2021;134:104538. DOI: <https://doi.org/10.1016/j.compbiomed.2021.104538>
19. Lu L, Hu W, Tian Z, et al. Developing natural products as potential anti-biofilm agents. *Chinese Medicine*. 2019;14:11. DOI: <https://doi.org/10.1186/s13020-019-0232-2>
20. Stan D, Enciu AM, Mateescu AL, et al. Natural Compounds With Antimicrobial and Antiviral Effect and Nanocarriers Used for Their Transportation. *Frontiers in Pharmacology*. 2021;12:723233. DOI: <https://doi.org/10.3389/fphar.2021.723233>
21. Vikram A, Jesudhasan PR, Pillai SD, et al. Isolimononic acid interferes with *Escherichia coli* O157:H7 biofilm and TTSS in QseBC and QseA dependent fashion. *BMC Microbiology*. 2012;12:261. DOI: <https://doi.org/10.1186/1471-2180-12-261>
22. Basak HK, Saha S, Ghosh J, et al. Sequence Analysis, Structure Prediction of Receptor Proteins and In Silico Study of Potential Inhibitors for Management of Life Threatening COVID-19. *Letters in Drug Design and Discovery*. 2021;19(2):108-122. DOI: <https://doi.org/10.2174/1570180818666210804141613>
23. Yeom JH, Kim HY, Lim JH, et al. A calcium channel blocker, manolide exerts an anti-allergic inflammatory effect through attenuating NF- κ B activity. *Immunopharmacology and Immunotoxicology*. 2021;43(6):799-805. DOI: <https://doi.org/10.1080/08923973.2021.1988101>
24. Han BN, Hong LL, Gu BB, et al. Natural Products from Sponges. In: Li Z, editor. *Symbiotic Microbiomes of Coral Reefs Sponges and Corals*. Springer; 2019:329-463. DOI: <https://doi.org/10.1007/978-94-024-1612-1>
25. Imchen M, Moopantakath J, Kumavath R, et al. Current Trends in Experimental and Computational Approaches to Combat Antimicrobial Resistance. *Frontiers in Genetics*. 2020;11:563975. DOI: <https://doi.org/10.3389/fgene.2020.563975>
26. Ayaz M, Ullah F, Sadiq A, et al. Synergistic interactions of phytochemicals with antimicrobial agents: Potential strategy to counteract drug resistance. *Chemico-Biological Interactions*. 2019;308:294-303. DOI: <https://doi.org/10.1016/j.cbi.2019.05.050>
27. Melander RJ, Basak AK, Melander C. Natural products as inspiration for the development of bacterial antibiofilm agents. *Natural Product Reports*. 2020;37(11):1454-1477. DOI: <https://doi.org/10.1039/D0NP00022A>
28. Malik JK, Nautiyal B, Kumar A. Doxycycline as potent inhibitor of COVID-19 main protease: in-silico docking approach. *European Journal of Biomedical and Pharmaceutical Sciences*. 2020;7(10):400-405.
29. Anthappagudem A, Enaganti S, Bhukya B. Integration of mutational and molecular docking studies: An in silico approach to assess the stability and binding potential of CYP3A4. *Journal of Applied Biology and Biotechnology*. 2022;11(1):161-170. DOI: <https://doi.org/10.7324/JABB.2023.110122>
30. Benvenuto D, Benedetti F, Demir AB, et al. Analysis of Three Mutations in Italian Strains of

SARS-CoV-2: Implications for Pathogenesis. *Chemotherapy*. 2021;66(1-2):33-37. DOI: <https://doi.org/10.1159/000515342>

31. Gowtham K, Pranav RJ. Milk dependent hemagglutination inhibition and calcium alginate immobilization of ricin isolated from *Ricinus communis* L. seeds. *Journal of Biological Studies*. 2022;5(4):705-713.

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