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ANTIBACTERIAL POTENTIAL OF *SPIRULINA PLATENSIS* PHYCOCYANIN PEPTIDES: A MULTIPLE MOLECULAR DOCKING ANALYSIS

Potensi Antibakteri dari Peptida Fikosianin *Spirulina platensis*: Analisis Multiple Molecular Docking

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ABSTRACT

Phycocyanin-derived peptides from Spirulina plantesis were screened as potential natural antibacterial agents. Simulated protein hydrolysis aimed for non-allergenic, non-toxic active peptides. Multiple ligand docking revealed nine peptides interacting with Pseudomonas aeruginosa's LpxC enzyme. These peptides showed diverse interaction values (-5.1 to -6.0 kcal/mol) compared to the native ligand BB-78485 (-9.7 kcal/mol). Notably, AC-AF, AC-APG, and AF-MA displayed -15.4, -15.3, and -14.7 kcal/mol affinities, respectively. Hydrophobic interactions and van der Waals forces played crucial roles in protein binding. ADME analysis identified YCL and ASYF peptides with poor pharmacokinetics, potentially hindering clinical application and further drug development. Overall, these phycocyanin peptides exhibit promise in antibacterial drug development, emphasizing their potential significance in the field.

Keywords: *phycocyanin peptides, Spirulina plantesis, in silico analysis, antibacterial, protein-ligand interactions*

ABSTRAK

Peptida yang berasal dari pigmen fikosianin *Spirulina plantesis* diskrining untuk mengidentifikasi peptida potensial sebagai kandidat antibakteri alami. Hidrolisis protein dengan enzim papain dan bromealin dilakukan secara in silico untuk mendapatkan peptida aktif yang bersifat non-alergen dan non-toksik, serta pendekatan multiple ligand docking (MLD) dilakukan untuk menjelaskan interaksi peptida-reseptor yang sinergis. Peptida fikosianin dievaluasi aktivitas anti-bakterinya sebagai peptida tunggal maupun campuran peptida terhadap enzim LpxC [UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc deacetylase] bakteri *Pseudomonas aeruginosa* dengan bantuan perangkat lunak AutoDock Tools 1.5.7. Sembilan peptida fikosianin menunjukkan nilai interaksi yang bervariasi antara -5,1 hingga -6,0 kkal/mol, dibandingkan dengan ligand natif BB-78485 (derivat sulfonamida sebagai agen antibakteri) yang memiliki nilai -9,7 kkal/mol. Hasilnya menunjukkan efek sinergis yang memberikan nilai afinitas pengikatan lebih tinggi dibandingkan interaksi peptida tunggalnya dan melebihi afinitas ligan natif, dimana AC-AF (Ala-Cys-Ala-Phe); AC-APG (Ala-Cys-Ala-Pro-Gly); dan AF-MA (Ala-Phe-Met-Ala) memiliki nilai afinitas -15,4; -15,3; dan -14,7 kkal/mol. Ikatan hidrofobik dan gaya van der Waals memainkan peran penting dalam ikatan pada situs aktif protein 2VES. Hasil studi ADME menunjukkan bahwa peptida YCL dan ASYF tidak memiliki sifat farmakokinetik

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yang baik. Hal ini dapat berdampak pada ketidakoptimalan penerapan klinis dan pengembangan obat antibakteri lebih lanjut. Secara keselutuhan, peptida aktif fikosianin dapat berpotensi dalam pengembangan obat antibakteri.

Kata Kunci: peptida fikoasianin, *Spirulina plantesis*, analisis in silico, antibakteri, interaksi protein-ligan

INTRODUCTION

The abundance of microalgae plays an important role in the fields of health and nutrition. Marine microalgae, including Cyanobacteria, are considered a source of various compounds such as fatty acids, carotenoids, polysaccharides, and pigmented proteins with diverse biological activities (Saharan et al. 2017). The metabolites in microalgae demonstrate various pharmacological activities, including anti-inflammatory, anti-diabetic, antioxidant, and anti-tumor properties (Selvaraj et al. 2017). Compounds from this blue-green microalga include provitamin A, vitamins C and E, essential amino acids, unsaturated fatty acids, and minerals, including iron, calcium, magnesium, manganese, sodium, potassium, phosphorus, and zinc (Akbarbaglu et al. 2022).

The bioactivity of phycocyanin peptides surpasses that of its native protein counterpart due to the liberation of active peptides. This phenomenon can be attributed to the heightened solubility and stability achieved through enzymatic hydrolysis of phycocyanin. One particular microalga that receives special attention for its utilization is *Spirulina platensis*. Recently, microalgal proteins have been evaluated as protein hydrolysates, encompassing peptides with potential bioactivities such as antioxidant, antifungal, anticancer, antibacterial, and others. Spirulina is a type of microalga rich in biopigments, one of which is phycocyanin, a complex blue pigment-protein compound consisting of α and β subunits (Grover et al. 2021). Several studies indicate that phycocyanin has the potential as an antioxidant, antibacterial, anti-inflammatory, immune system stimulant, anti-proliferative agent in cancer, and anti-diabetic agent (Koyande et al. 2019; Stadnichuk & Tropin 2017; Zheng et al. 2013). The bioactivity of phycocyanin peptides surpasses that of its native protein counterpart due to the release of active peptides. This enhancement can be attributed to the increased solubility and stability resulting from the enzymatic hydrolysis of phycocyanin (Xu et al. 2018; Silva et al. 2021).

Research on bioactive compounds and antibiotic resistance from peptides is rapidly evolving due to the pressing need to tackle antibiotic resistance in bacteria. The rise of antibiotic resistance in hospitals, communities, and the environment has been a major concern (Lin et al. 2023; Nadgir and Biswas 2023). Bacteria have the ability to adapt to antibiotics due to their swift evolution, and the inappropriate use of antibiotics has led to the development of multidrug-resistant bacteria (Serwecińska 2020). The development of new antibiotics and other antimicrobials continues to be a pressing need in humanity's battle against bacterial infections. Phytochemicals have been proposed as an alternative to complement antibiotics due to their variation in genetic, and the pharmaceutical industry has developed diverse antibiotics to address resistance issues (Suganya et al. 2022). However, the curing proportion of patients was comparatively less, making bacterial infections worse. Therefore, research on bioactive compounds and antibiotic resistance from peptides is crucial to develop new therapeutic strategies and future prospects to tackle antibiotic resistance in bacteria.

In recent years, the healthcare landscape has witnessed remarkable progress, yet it has been accompanied by a pressing concern - antibiotic resistance. The escalating challenge of antibiotic resistance has emerged as a global threat, characterized by the increasing resilience of bacteria to conventional antibiotic treatments. Notably, phycocyanin derived from *S. platensis* has demonstrated noteworthy antibacterial efficacy against both gram-positive and gram-

negative bacteria. Nevertheless, it is worth noting that the exploration of peptides derived from *S. platensis* remains a relatively underexplored avenue in scientific research, despite its promising potential in the context of combating antibiotic resistance (Sadeghi et al. 2018). Phycocyanin is the most abundant protein in Spirulina, comprising 20% of its dry biomass (Romay et al. 2005). Additionally, phycocyanin from S. platensis has antibacterial activity against both gram-positive and gram-negative bacteria (Izadi & Latifi 2022). Although the protein content of this microalga has been extensively documented, the study of its peptide constituents has not been explored in depth. This lack of knowledge poses a significant challenge since peptides often possess unique bioactive properties that have the potential to be utilized in various applications, including the development of novel pharmaceuticals and functional foods (Bianco et al. 2022). Research on phycocyanin peptides has led to the development of docking methods to understand the molecular interactions between phycocyanin peptides and bacterial targets. Docking is a computational structure-based technique used to predict binding between small molecules (ligands) and target proteins (Zhou et al. 2018). Computational modeling provides insights into the molecular recognition mechanisms between the related target macromolecules and compounds with biological activity. In this context, multiple ligand docking (MLD) has become a valuable approach for studying the effects of phycocyanin peptides on their antibacterial bioactivity (Li et al. 2023).

The computational method MLD is employed to study the interactions between multiple ligands and the target protein. This approach is advantageous in understanding the complexity of interactions between phycocyanin peptides and bacterial targets, as it identifies the most stable bindings that contribute to the peptides' antibacterial activity. Additionally, MLD can investigate how structural variations in phycocyanin peptides can affect their interactions with the bacterial target. It can also identify how small molecules binding to the same target can be combined or enlarged into larger compounds with improved affinity (Codina et al. 2023).

This method proves highly valuable for studying mixtures of phycocyanin peptides, allowing exploration of their synergistic or antagonistic effects on the target receptor. The advantages of Multiple Ligand Docking (MLD) in investigating phycocyanin peptides lie in its ability to provide insights into the binding affinity and selectivity of these peptides toward their target receptor (Raghavendra et al. 2015; Ban et al. 2018). By considering the conformational flexibility of both ligands and receptors, MLD aids in identifying the most advantageous binding configurations and predicting the relative potency of various phycocyanin peptides. This information proves beneficial for the rational development of phycocyanin-based antibiotics.

Studies have shown that phycocyanin peptides can function as antibacterial agents by inhibiting the formation of bacterial cell walls or disrupting cell membrane functions (Effendi et al. 2020; Safari et al. 2022). However, the use of multiple phycocyanin peptides to interact with bacterial targets is currently restricted. To address this issue, this research utilizes the MLD approach to examine how phycocyanin peptides interact with bacterial protein targets. This has the potential to pave the way for new treatments for bacterial infections and the development of more effective antibacterial strategies. Nevertheless, the application of multiple phycocyanin peptides for bacterial target interactions remains notably constrained. Hence, this study aims to employ the Multiple Ligand Docking (MLD) approach to investigate the interactions of phycocyanin peptides, with a specific focus on bacterial targets, particularly through the inhibition mechanism of N-acetyl glucosamine deacetylase (LpxC). Numerous studies that demonstrate the LpxC enzyme's potential as an antibacterial therapeutic target corroborate the reasoning behind targeting it in *Pseudomonas aeruginosa*. The first committed step in the production of the lipid A component of lipopolysaccharides (LPS), which is a pivotal component of the cell wall in gram-negative bacteria, is catalyzed by the metalloamidase enzyme LpxC. LPS assumes a critical role in preserving the structural integrity and membrane functionality of bacterial cells (Erwin, 2016). Research has

confirmed LpxC's molecular validity as a target for innovative antibiotic medications, highlighting its importance in relation to *Pseudomonas aeruginosa* infections (Niu et al. 2023).

Pseudomonas aeruginosa LpxC was verified as a target for novel antibiotic medicines (Niu et al. 2023). The study also stressed the significance of developing inhibitors against this Gram-negative bacteria, which is therapeutically significant. These collective findings emphasize the importance of LpxC as a promising target for the creation of new antibiotic medications against *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* was selected as the research subject due to its significance as a human bacterial pathogen, especially among immunocompromised patients. (Krause et al. 2019). This approach forms a part of the ongoing effort to explore innovative alternatives in the development of effective antibiotics and combat the escalating antibiotic resistance. Thus, this research may pave the way for novel possibilities in bacterial infection treatment and the development of more efficacious antibiotic strategies.

MATERIALS AND METHODS

The Chemoinformatics Properties of Phycocyanin Peptides

To obtain the structure of phycocyanin (consisting of α and β subunits), Uniprot (https://www.uniprot.org/) was used. To obtain the phycocyanin sequence from *Spirulina platensis*, one can select the accession number "P72509 (C-phycocyanin alpha subunit) and P72508 (C-phycocyanin beta subunit)" associated with this species. The phycocyanin sequence was then replicated on BIOPEP website (https://biochemia.uwm.edu.pl/en/start/) to enzymatically hydrolyze it using papain (EC 3.4.22.2) and bromelain (EC 3.4.22.32) enzymes. Various online servers were utilized to assess the chemoinformatics properties of the tested phycocyanin peptides, including PeptideRanker to identify peptides with activity, ToxinPred for peptide toxicity evaluation, and AllerTop v2.0 (https://www.ddg-pharmfac.net/AllerTOP/) to predict allergenicity. Bioactive peptides typically fall within the range of ≥ 0.7; however, 0.8 is chosen as the minimum threshold for PeptideRanker to minimize false positive results (Mooney et al., 2012). The two previously non-toxic and non-allergenic peptides were visualized using Avogadro.

Molecular docking

The crystal structure of the metalloprotein complex LpxC *from Pseudomonas aeruginosa* (PDB ID: 2VES) was obtained from the Protein Data Base (https://www.rcsb.org/pdb). The protein and ligand (peptide) were prepared for AutoDock and AD_{Zn} using AutoDock Tools 1.5.7 software, which added hydrogen atoms and partial charges computed using Gasteiger's method (Asiamah et al. 2023; Santos-Martins et al. 2014). AutoDockTools provides several tools to add hydrogen atoms (Edit→ Hydrogen → Add Polar Only) and partial charges (Edit → Charges → Compute Gasteiger) to the molecule. Rotation and grid box settings were used to determine the ligand-binding site, which was based on the original ligand that was bound to the protein macromolecule upon download, specifically the inhibitor BB-78485. The grid box is created by selecting the grid tab > grid box > center on ligand on the x, y, and z axes, consecutively 18 Å, 28 Å, and 22 Å. The distance used for the x, y, and z dimensions is 0,375 Å. Following the adjustment of the grid box size, the file is saved in the grid.txt format. This process involves navigating to the "File" tab and selecting the "Output Grid Dimension File" option. Subsequently, the PyMOL 2.5.2 is utilized to ascertain the RMSD values derived from ligand simulations.

To validate the method, the metalloprotein zinc log was recalculated with the native ligand, which should yield a Root Mean Square Deviation (RMSD) value less than 2Å. Multiple ligand docking (MLD) was also conducted on the phycocyanin peptides to examine the differences in interactions between ligands and multiple ligands tethered to the protein. The protein-ligand complex structures were visualized using PyMOL 2.5.2 and BIOVIA Discovery Studio Visualizer. Multiple ligand complexes with the protein were generated using Chimera X (Seeliger & De Groot 2010). BIOVIA

Discovery Studio Visualizer was used to inspect the 3D complex structure of both single and multiple ligand complexes and to analyze the interactions between amino acid residues formed between the protein and ligand.

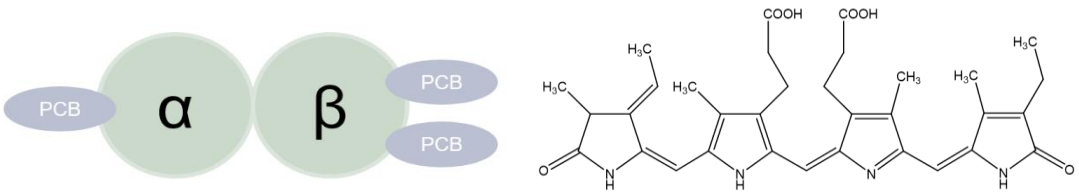
ADME Analysis of Phycocyanin Peptides

The crystal structure properties (Absorption, Distribution, Metabolism, and Excretion) of nine active phycocyanin peptides were analyzed in silico using SwissADME (http://www.swissadme.ch (Senadheera et al. 2022)), which allows users to draw 2D structure of the peptides. SwissADME uses ADME characteristics (absorption, distribution, metabolism, and excretion) of compounds to estimate pharmacokinetic indicators and drug-likeness. The structures of the hydrolyzed peptides were converted into SMILE (Simplified Molecular Input Line Entry System) files (Ji et al. 2022). Predicting the pharmacokinetic and pharmacodynamic features of peptides requires careful consideration of the selected ADME attributes and drug-likeness indicators. Researchers can find promising drug candidates with the best ADME characteristics and drug-likeness by examining these attributes. These peptides can then be developed further for medicinal uses (Turabi et al. 2022).

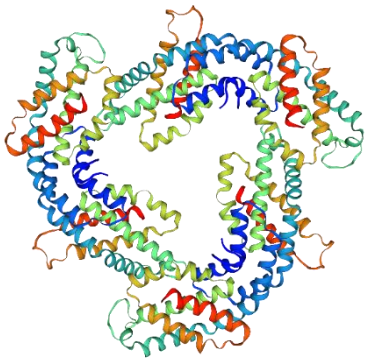
RESULT AND DISCUSSION

Molecular Docking

Figure 1 shows the visual representation presented illustrates the three-dimensional configuration of phycocyanin, a pigment produced by the cyanobacterium *S. platensis* (PDB ID: 1GH0). Within the intricate structure of phycocyanin, one can observe both a linear tetrapeptide chain and a cyclic peptide chain. The diagram aims to highlight specific features of phycocyanin, such as its conjugated bilin ring system, facilitating a deeper understanding of its molecular composition and potential biological roles (Yuan et al. 2022). Phycocyanin is a pigment-protein made up of one alpha (α) polypeptide and one beta (β) polypeptide, with a molecular weight between 10-21 kDa, creating a monomer. The α subunit bonds covalently to one phycocyanobilin (PCB), while the β subunit carries two phycocyanobilins. Phycocyanobilin is a bilin pigment that comes from phytochrome with a tetrapyrrole open-chain structure that attaches to the protein through a thioether bond, giving phycocyanin its blue color (Pez Jaeschke et al. 2021; Yuan et al. 2022).



(A)



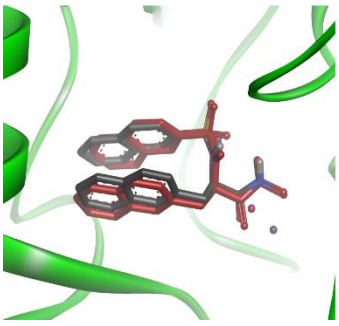
(B)

Figure 1. The chemical structure of phycocyanin from Spirulina platensis (A) and the monomeric αβ structure modeled using the SWISS-MODEL webserver (B).

The antibacterial properties of peptides in phycocyanin were tested through the metalloprotein-peptide docking method. To evaluate their effectiveness against Pseudomonas aeruginosa bacteria, phycocyanin peptides from Spirulina platensis were tested for their inhibitory activity against the LpxC enzyme. This enzyme is essential in the formation of lipid A, a critical component of the structure of Gram-negative bacterial cell walls. By interfering with bacterial cell wall biosynthesis, LpxC enzyme inhibition was shown to be an effective mechanism for inhibiting bacterial growth. Additionally, lipopolysaccharide (LPS) synthesis, which is a necessary component of Gram-negative bacterial cell walls, is initiated by this enzyme (Kalinin & Holl, 2016).

To validation the method, the native ligand inhibitor BB-78485 was utilized, with coordinates x, y, and z set at 57.657 Å, 37.871 Å, and 16.782 Å. The spacing parameters were set at 0.375 Å for x, y, and z at 18 Å, 28 Å, and 22 Å. The binding affinity energy between the ligand BB-78485 and the metalloprotein LpxC amounted to -9.703 kcal/mol, with an RMSD of 0.780 Å, which demonstrated the predicted binding structure's accuracy, as the RMSD value was under 2 Å (Figure 2) (Shi et al. 2022). The RMSD is a heuristic measure of how similar the real ligand position in the receptor is to the docking ligand's estimated position. The better the value, ideally, the lower the RMSD score (López-Camacho et al. 2016). The two overlapping molecular structures in this illustration represent the initial pose of the native ligand and the final position after a molecular redocking simulation. By conducting a redocking simulation, allows an evaluation of the docking method's ability to accurately reproduce the binding position of the ligand at the active site of the enzyme or target receptor. This verification ensures that the simulation method effectively predicts the interactions between the ligand and the target, thereby enhancing the reliability and relevance of the obtained results in research or drug development (Akabli et al. 2019; Umar et al. 2021).

Based on the validation results, it appears that the process of peptide docking can be applied to the test peptides obtained from phycocyanin peptides. These peptides were identified through chemoinformatics and are listed in Table 1. After papain hydrolysis, only 2 out of 77 subunit α peptides and 3 out of 109 subunit β peptides were produced. Bromealin hydrolysis yielded 1 out of 88 subunit α peptides and 1 out of 109 subunit β peptides. Papain-bromealin hydrolysis resulted in 1 out of 109 subunit α peptides and 2 out of 126 subunit β peptides with Peptide Ranker scores of ≥ 0.70, meeting the criteria for non-toxicity and non-allergenicity. The possibility that a peptide sequence is bioactive was ranked using Peptide Ranker scores, which ranged from 0.0 (very unlikely) to 1.0 (very likely). Peptides with scores ≥0.70, which were thought to be potentially bioactive and hadn't been reported before, were the study's main emphasis (Pearman et al. 2020). It is important to note that these results were obtained without any harmful effects.



The native ligand in stick mode:

- Black: Initial pose of the native ligand
- Red: Re-docked pose of the native ligand

Figure 2. The shape overlay of the initial pose and the pose after molecular redocking simulation.

The results obtained from the molecular docking study in Table 2 indicate that among the hydrolyzed peptides AC, MA, YCL, AF, PG, TF, and YF, they exhibit a reasonably strong binding affinity to the LpxC enzyme of *Pseudomonas aeruginosa* bacteria, with binding energy values ranging from -5.1 to -6.0 kcal/mol, compared to BB-78485 (-9.7 kcal/mol). The variation in binding energy values indicates that the hydrophobicity, charge, size, and shape of individual amino acids can influence the affinity of peptide-peptide interactions. This implies that these factors, which impact a peptide's overall characteristics, can also influence its binding efficiency to other peptides (Cherry et al. 2014; Du et al. 2019). However, weak binding affinities were observed for the peptides APG and ASYF. YF achieved the highest docking score among the peptides but fell short of surpassing the native ligand (BB-78485). Despite having fewer hydrogen bonds, BB-78485 showed a higher number of non-covalent interactions, including hydrophobic and electrostatic bonds, compared to YF. The hydrophobic interactions of BB-78485 play a more significant role in enhancing it's binding affinity. Energetically, these weak intermolecular interactions, particularly hydrophobic bonding, play a crucial role in stabilizing the ligand at the protein interface (Varma et al. 2010). Hydrophobic interactions can lower enthalpy by reducing contact with polar water through the formation of non-polar bonds, resulting in increased protein-peptide affinity (Li et al. 2021).

Table 1. Selected Peptides Resulting from Papain, Bromelain, and Papain-Bromelain Enzymatic Hydrolysis

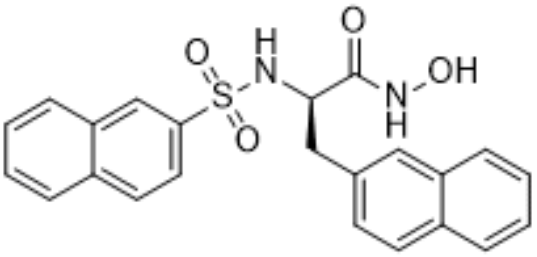
Polypeptide	Papain	Bromealin	Papain-Bromealin	Bioactivity	Toxicity	Allergenicity
Subunit α	AF YCL	MA	YCL	Active peptide	Non-toxic	Non-allergen
Subunit β	AC APG ASYF TF	YCL	YF PG	Active peptide	Non-toxic	Non-allergen

Table 2. Docking Outcomes of Active Phycocyanin Peptides with Metalloprotein 2VES

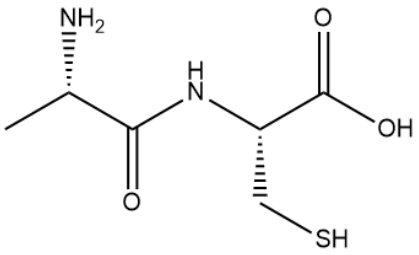
Ligand	Affinity (kcal/mol)	Hidrogen bond	Hydrophobic bond	Electrostatic	Other	Van der Waals	Unfavorable	Total
BB-78485	**-9,7**	**4**	**11**	**0**	**3**	**15**	**4**	**37**
AC	-5,1	6	1	3	1	5	3	19
MA	-5,4	7	2	0	1	7	2	19
AF	-5,7	6	1	3	2	6	2	20
TF	-5,9	7	0	0	2	7	2	18
YF	-6,0	5	3	0	0	11	2	21
PG	-5,7	8	1	3	1	8	2	23
APG	-4,7	6	3	3	0	5	3	20
ASYF	-3,7	4	7	0	0	8	3	22
YCL	-5,6	4	5	0	1	14	3	27

The binding capability of peptides to the active site of 2VES is influenced by the chemical properties of the amino acid side chains in their vicinity. Among the peptides containing the amino acid phenylalanine, namely AF, TF, and YF, variations in binding affinity are observed. This variation can be attributed to the differences in the number of interactions involved. Notably, YF exhibits higher binding affinity than AF and TF due to

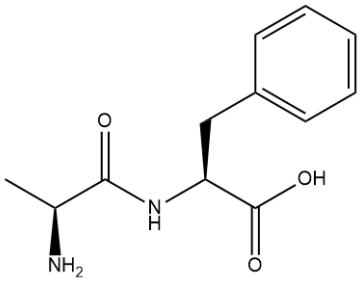
it's increased interaction count. Furthermore, YF incorporates residues with ring structures (see Figure 3), which typically contribute significantly to phi-phi stacking interactions (hydrophobic). Consequently, YF attains a higher binding energy. In comparison to TF, the structure of AF, with alanine that is relatively nonpolar, lacks hydroxyl (-OH) functional groups necessary for hydrogen bonding with the 2VES protein. Consequently, the resulting affinity is lower than that of TF, as AF features fewer hydrogen bonds.



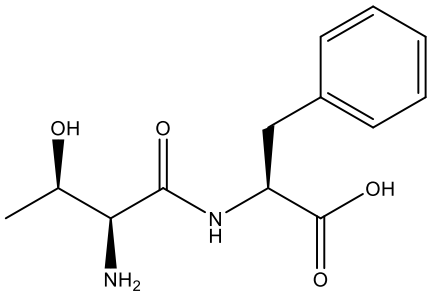
Native Ligand (**BB-78485**)



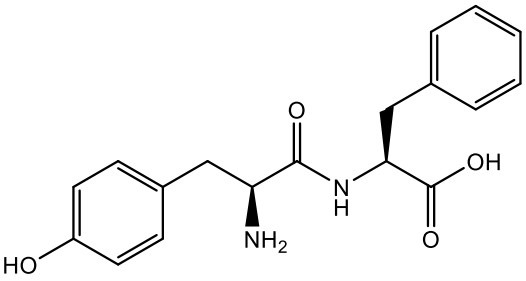
AC (Ala-Cys)



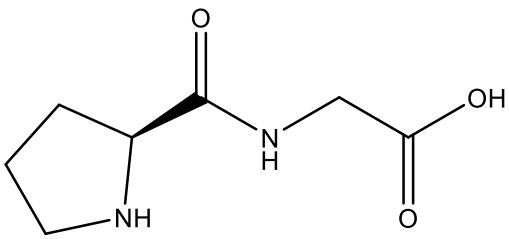
AF (Ala-Phe)



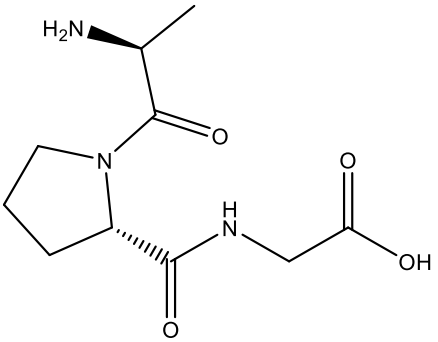
TF (Thr-Phe)



YF (Tyr-Phe)



PG (Pro-Gly)



APG (Ala-Pro-Gly)

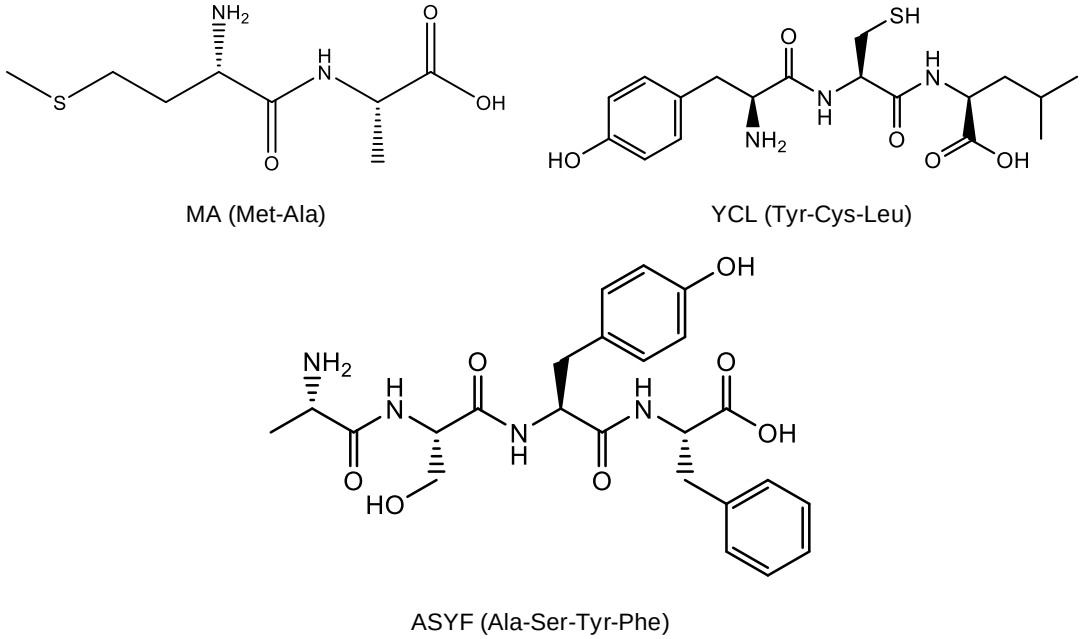


Figure 3. Structure of various phycocyanin peptides resulting from hydrolysis.

In studying the interaction between numerous ligand molecules and a protein's active site, the MLD method was utilized (H. Li et al. 2011). Table 4 presents the docking scores and 3D visualizations of the protein's interactions with multiple ligands 2VES. The results indicate that the peptides AC-AF demonstrate greater affinity compared to other multiple ligands, scoring -15.4. Following closely are AC-APG and AF-MA, with scores of -15.1 and -14.7, respectively. It is worth noting that various factors, such as ligand size, ligand-metalloprotein interactions, and ligand conformations, can impact these results. It is possible for the ligands to form stronger interactions with the metalloprotein during docking, leading to lower scores and higher affinities (Zuo et al. 2017). When multiple ligands are introduced, the potential interaction space expands and the system complexity increases. This expansion enhances the probability of discovering stable and optimal conformations during docking, leading to high affinities. However, it is important to note that low affinities are almost exclusively obtained when multiple ligands bind to YCL, despite YCL exhibiting reasonably high affinity during single-ligand interactions.

The search results reveal many articles discussing how molecular docking is used to explore the potential effectiveness and specificity of various ligands. In one study, natural compounds baicalein and cubebin are examined for their combined impact on blocking the main protease of SARS-CoV-2. This study identifies promising compounds, with multi-ligand molecular docking demonstrating their intriguing drug-like properties and lack of toxicity. Additionally, the study investigates the interaction energy between the main protease and the ligands, providing valuable insights into potential interactions among these molecules (Li et al. 2023). Similarly, to the research conducted by Noshad et al. (2023), this study investigates the synergistic effect antimicrobial activities of the essential oils from *Coriandrum sativum seed* (CO) and *Cuminum cyminum* (CUM). When Linalool and Carvone are combined, there is a notable enhancement in the docking score for receptor 1KZN, improving from -9.311 (Carvone alone) to -9.081. This suggests a synergistic interaction between the two ligands and this is consistent with its in vitro results against several bacteria.

The data in the table below reveal that the amino acid phenylalanine (F) present in multiple ligands YF, TF, and AF results in relatively low affinities. The inclusion of additional ligands with amino acid F may

modify the optimal conformation of YCL when binding to the metalloprotein, which can reduce the complex's affinity. Additionally, ligands may compete for binding to the same active site on the metalloprotein (Vauquelin & Charlton 2013).

In the context of ligand competition above, this can affect how a compound is absorbed, distributed, metabolized, and excreted by the body. If one ligand has a stronger attraction to a metalloprotein than another, such as YF (-6.0) compared to YCL (-5.6), YF may outcompete YCL for binding to the metalloprotein. This can decrease the availability of YCL ligands to interact with their intended biological targets, potentially affecting the absorption and distribution of the compound within the body's tissues (Ekins et al. 2010). It is important to note that the metabolism and elimination rate of a compound can be affected by ligand competition. The extent of this impact on ADME will depend on the chemical and pharmacokinetic properties of the peptides involved, as well as the biological system (Devi et al. 2023).

In line with the research conducted by Ramirez-Acosta et al. (2022), the molecular docking results guided the selection of peptides with high affinity for the Receptor Binding Domain (RBD). The identification of the most common amino acids involved in RBD recognition provided insights for designing novel peptides, emphasizing the number of hydrogen bonds formed. In addition to binding affinities, the consideration of ADME (Absorption, Distribution, Metabolism, and Excretion) physical properties is crucial for drug development. The peptides were observed to be nearly neutral at physiological pH, and their solubility in aqueous media further enhances their potential as drug candidates. This correlation highlights the importance of not only strong binding to the target (affinity) but also favorable ADME properties, ensuring potential success in drug development.

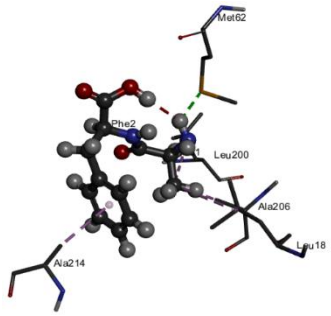
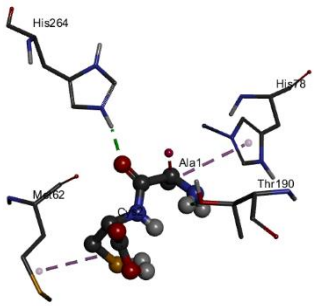
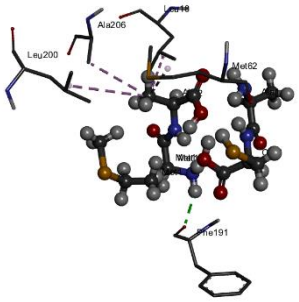
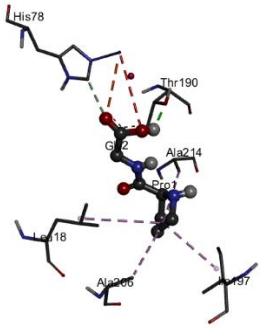
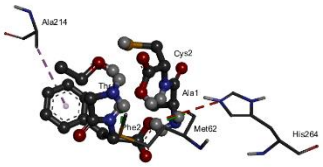
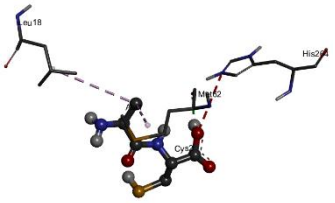
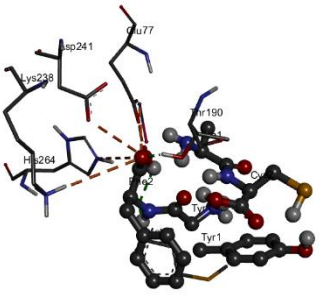
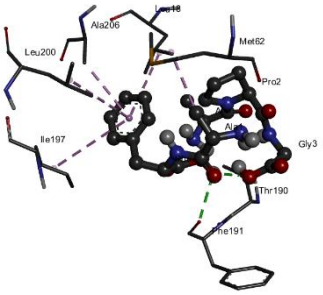
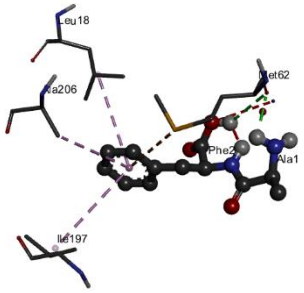
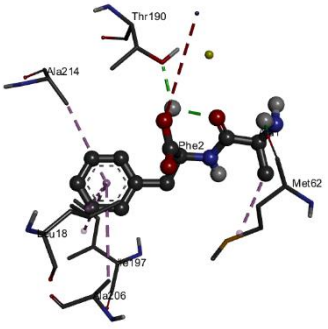
Table 3. Multiple Ligand Affinities YCL on Metalloprotein 2VES

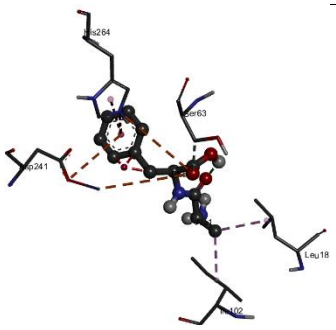
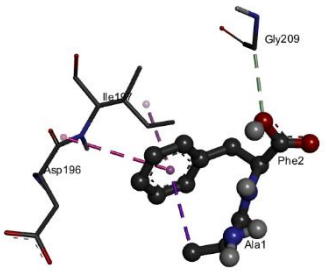
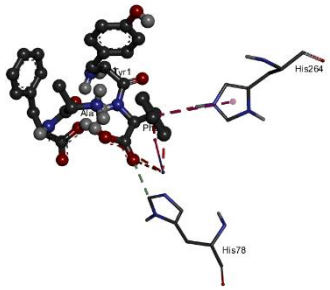
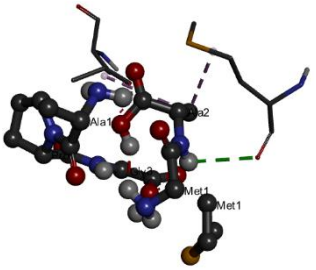
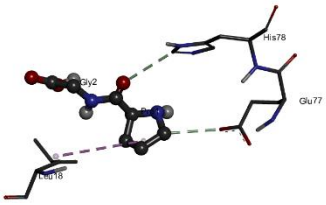
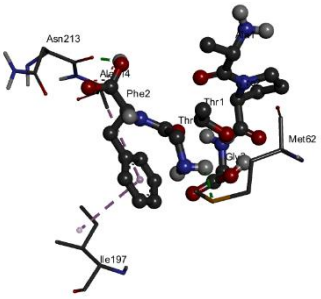
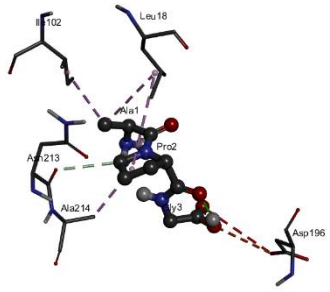
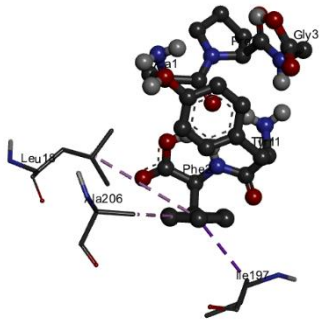
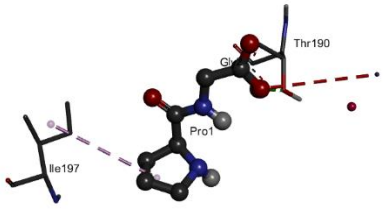
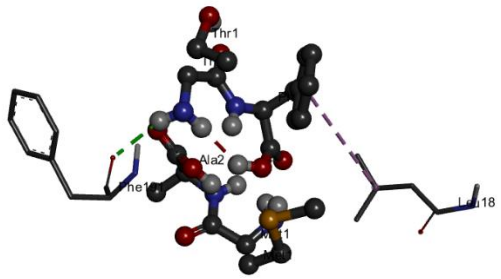
Peptide	Affinity (kcal/mol)
YF-YCL	93,71
TF-YCL	-0,25
AF-YCL	-4,7
APG-YCL	-5,7
PG-YCL	-7,9
AC-YCL	-8,3
MA-YCL	-9,6

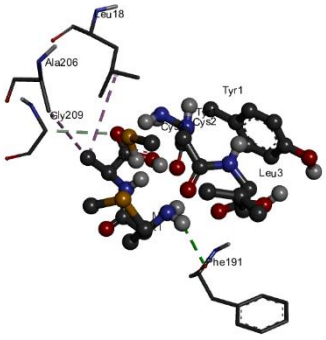
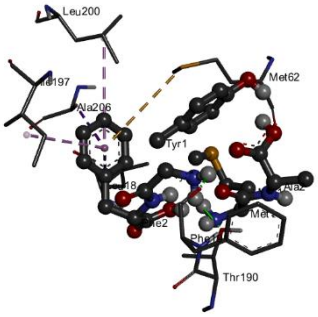
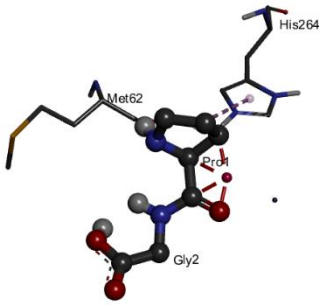
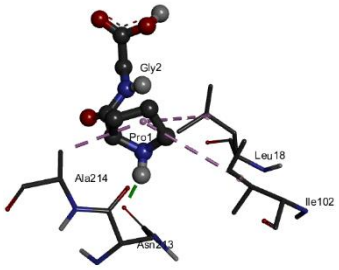
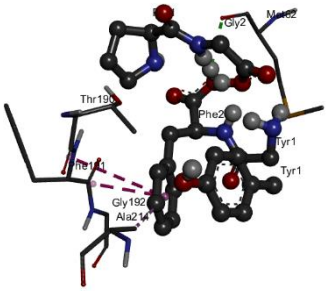
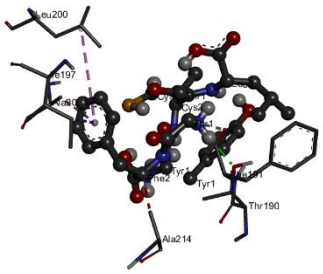
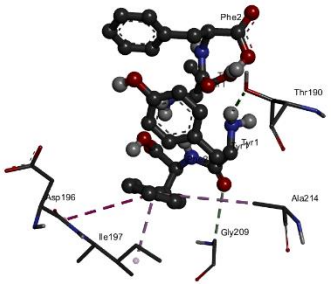
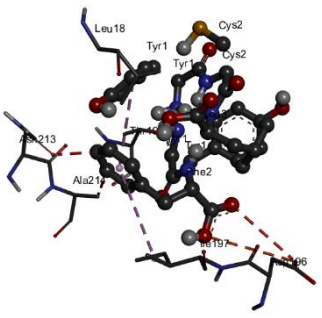
Pharmacokinetics in Silico (Drug Similarity) (ADME)

ADME involves a sequence of procedures that demonstrate how a compound interacts with the body. SwisssADME is a useful tool for measuring multiple aspects related to the physical and chemical properties, pharmacokinetics, drug similarity, and other relevant factors of one or more molecules (Daina et al. 2017). Lipinski's rule uses the "Rule of Five" to assess the pharmacokinetic properties of lead molecules for effectiveness and safety (Lipinski et al. 2001).

Table 4. 3D MLD visualization of phycocyanin bioactive peptide with 2VES

Multiple Peptide	Affinity (kcal/mol)	Multiple Peptide	Affinity (kcal/mol)
			
AC-AF	-15,4	**AC-APG**	-15,1
			
AC-MA	-13,3	**AC-PG**	-12,4
			
AC-TF	-14,5	**AC-YCL**	-8,3
			
AC-YF	-11,6	**AF-APG**	-12,2
			
AF-MA	-14,7	**AF-PG**	-14,4

			
AF-TF	-11,7	**AF-YCL**	-4,7
			
AF-YF	-8,8	**APG-MA**	-13,6
			
APG-PG	-12,6	**APG-TF**	-12,9
			
APG-YCL	-5,7	**APG-YF**	-9,0
			
MA-PG	-12,9	**MA-TF**	-14,3

			
MA-YCL	-9,6	**MA-YF**	-11,6
			
PG-TF	-12,5	**PG-YCL**	-7,9
			
PG-YF	-11,7	**TF-YCL**	-0,25
			
TF-YF	-7,2	**YCL-YF**	93,71

In this study, ASYF and YCL peptides had the number of rotatable bonds (ROTB), number of hydrogen bond donors (HAD), and topological polar surface area (TPSA) that exceeded the rule limits indicating poor oral bioavailability (Ji et al. 2020). In **Table 5**, it is evident that out of the nine estimated peptides from the phycocyanin protein hydrolysate, seven of them appropiate to the Lipinski criteria. The qualitative potential of these peptides to be turned into oral medications was evaluated based on their bioavailability and Lipinski values, with a focus on their structural features and biological availability (Senadheera et al. 2022). The ability of a drug to spread and circulate in the body is measured by its bioavailability, which is considered ideal if it has a score

above 0.25 (Martin 2005). Thus, ASYF is considered to have poor bioavailability when used as an oral drug.

The Kp value, which is the measure of skin permeability in cm/s, determines how easily molecules can enter the skin (Ibrahim et al. 2021). The log Kp value reveals the potential danger of a substance to the skin. The peptides that were tested had low Kp values, ranging from -9.49 to -11.94 cm/s, indicating that molecules have limited ability to penetrate the skin. The primary factor that affects this parameter is the size of the molecule, with larger molecules experiencing more difficulty in penetrating the skin layer (Ojuka et al. 2023). Based on research conducted by Aziz (2023), where ZINC20 compounds have values ranging from -7.93 to -9.69 indicating that all molecules are considered to have lower skin penetrating ability. Gastrointestinal adsorption (GIA) refers to the ability of a compound to be absorbed through the gastrointestinal tract (stomach and intestines) after being consumed by mouth (Olasupo et al. 2021). This absorption ability is important because it affects how effectively a compound can enter the bloodstream and reach its target in the body. AC, ASYF, and YCL peptides have low GIA, resulting in inefficient absorption in the stomach and intestines.

Table 5. ADME Analysis

Peptide	Physicochemical						Lipofilicity	Drug Compatibility		Pharmakokinetic		
	Molecule weight (g/mol)	ROT B (n)	HBA (n)	HAD (n)	Log S (ESOL)	TPSA (Å²)	Log $P_{o/w}$	Bioa-vailibilty	Lipinski	GIA	Log Kp	P-gp Sub-strate
Terms	<500	<10	<10	<5	-	<140	<5	-	-	-	-	-
AC	192.24	5	4	3	1,4	131,22	-1,22	0,55	Yes (0)	Low	-9,84	No
MA	220,29	7	4	3	1,45	117,72	-0,67	0,55	Yes (0)	High	-10,12	No
AF	236,27	6	4	3	0,39	92,42	0,05	0,55	Yes (0)	High	-9,49	No
TF	266,29	7	5	4	0,68	112,65	-0,52	0,55	Yes (0)	High	-10,12	No
YF	328,36	8	5	4	-0,66	112,65	0,78	0,55	Yes (0)	High	-10,20	No
PG	172,18	4	4	3	1,76	78,43	-1,17	0,55	Yes (0)	High	-10,06	No
APG	243,26	6	5	3	1,16	112,73	-1,32	0,55	Yes (0)	High	-10,17	No
ASYF	486,52	15	8	7	0,25	191,08	-0,63	0,17	No (2)	Low	-11,94	Yes
YCL	397,49	12	6	5	-0,37	180,55	0,31	0,55	Yes (0)	Low	-10,20	No

CONCLUSION

Based on the docking results, bioactive phycocyanin peptides from hydrolysis processes have the potential as antibacterial agents against *Pseudomonas aeruginosa*. The research findings show that the nine phycocyanin peptides AF, AC, APG, MA, PG, YF, TF, YCL, and ASYF interact with binding values ranging from -5.1 to -6.0 kcal/mol compared to the native ligand BB-78485 (sulfonamide derivative) with a value of -9.7 kcal/mol. MLD was employed to identify potential synergies within the ligand peptides, presenting an innovative approach to uncover novel antimicrobial agents. The docking results offer valuable insights into the impact of ligand combinations and their individual binding affinities to diverse receptors. respectively. Hydrophobic interactions and van der Waals forces play an important role in binding to the active site of the 2VES protein, including His78, Leu18, Ala214, His237, and His264. The ADME analysis results show that the peptides YCL and ASYF do not have good pharmacokinetic properties, which potentially hindering clinical application and further drug development. This research indicates the need for further investigation into understanding the synergy of peptides in both in vitro and in vivo settings, as well as the exploration of other types of peptides combined with phycocyanin peptides. Such endeavors could contribute to the development of novel combination antimicrobials. Overall, the MLD method

shows potential in the the identification and development of antibacterial drug discovery from peptides phycocyanin.

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