International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Special Issue 4, 2022

Original Article

IN SILICO STUDY OF BIOACTIVE COMPOUNDS FROM SUNGKAI (*PERONEMA CANESCENS*) AS IMMUNOMODULATOR

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Received: 15 Jul 2022, Revised and Accepted: 30 Aug 2022

ABSTRACT

Objective: This study aims to predict a bioactive compound from *Peronema canescens* (PC) with mechanisms inhibitor interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) potential as an immunomodulatory using *in silico* approach.

Methods: Autodock 4 was used to accomplish computer-assisted drug design with molecular docking simulation to discover binding energy, inhibition constant, and interactions with an amino acid in bioactive compounds from PC against IL-6 and TNF-α receptors. Lipinski predicts the drug-likeness of a bioactive compound for the oral route of administration. ADMET profiling of bioactive compounds to predict pharmacokinetic properties with pkCSM ADMET.

Results: The results showed that the best binding energy, inhibition constant, and interactions with an amino acid of peronemin C1 against IL-6 and TNF-α receptors were (-7.19 kcal/mol; 5.39 nM; Arg 179, Arg 182, Gln 175), and (-8.86 kcal/mol; 320.42 nM; Tyr 119, Tyr 59, and Gly 121), respectively. All bioactive compounds from PC met Lipinski's rule of five requirements for oral administration. ADMET prediction results all bioactive compounds from PC are non-mutagenic, except peronemin D1 is mutagenic.

Conclusion: The peronemin C1 bioactive compounds from PC have good immunomodulatory potential, effectively inhibiting human IL-6 and TNF-α receptors using *in silico* approach.

Keywords: Sungkai, Peronemin, IL-6, TNF-α, Immunomodulatory, COVID-19, *In silico*

DOI: https://dx.doi.org/10.22159/ijap.2022.v14s4.PP33 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

The developments in understanding the pathogenesis of COVID-19 disease reveal that cytokine release syndrome (CRS), Increased levels of interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) are associated with illness severity. Therefore, it has been advised that severe COVID-19 patients may be saved by treating CRS [1]. Furthermore, modification in IL-6 levels may reduce COVID-19's length and intensity. Therefore, the Food and Drug Administration (FDA) has authorized two categories of IL-6 inhibitors, namely, anti-IL-6 monoclonal antibodies (mAbs) (e. g., siltuximab) and anti-IL-6 receptor mAbs (e. g., tocilizumab, sarilumab). These drugs have been tested on COVID-19 patients with systemic inflammation [2]. In addition, TNF-α is one of the proinflammatory cytokines commonly increased in acute lung damage, producing CRS and enhancing the interaction between SARS-CoV-2 and angiotensin-converting enzyme 2 (ACE2). Therefore, TNF-α inhibitors may be an appropriate therapeutic choice for the slower progression of severe SARS-CoV-2 infections [1].

White blood cells and other parts of the immune system work together to protect the body against infections [3]. The immune system consists of innate immune immunity and adaptive immune immunity. First, innate immunity defends against infections and stimulates adaptive immunity to strengthen defenses. Innate immunity is immunity with the shortest response time. It comprises neutrophils, monocytes, dendritic cells, and macrophages. However, T and B cells are involved in adaptive immunity [4]. In addition, several cytokines, including proinflammatory cytokines such as IL-6 and TNF-α, are essential mediators of the immune response. As intercellular messenger molecules, cytokines have multiple activities, including boosting phagocyte migration and coordinating the early responses of lymphocytes, monocytes, macrophages, and dendritic cells under inflammatory situations [5].

Regarding the role of cytokines in pathogenesis, it is necessary to develop individualized immunomodulatory therapies. Inhibition of IL-6 and TNF-α, as well as activation of the complement system, are some of these therapeutic targets [6]. The soluble mediator IL-6 with a multifaceted influence on hematopoiesis, inflammation, and immune response [7]. Human IL-6 has 212 amino acids, with a signal peptide of 28 amino acids, and its gene has been found on chromosome 7p21. While glycosylation raises its size to 21–26 kDa and IL-6's core protein is 20 kDa [7]. TNF-α is a pleiotropic cytokine that functions as an essential function in disease pathogenesis and homeostasis. TNF-α induces inflammation, activates vascular endothelium, coordinates immune cell recruitment into tissues, and promotes tissue degradation [8].

Herbal medicine is acknowledged to have an essential role in controlling infectious diseases. Furthermore, several studies have shown that a combination of herbal medicine and modern medicine can relieve symptoms and improve the quality of life in COVID-19 patients [9]. Furthermore, In China and South Korea, herbs that are frequently used to treat COVID-19 include *Citri reticulatae* pericarpium, *Glycyrrhizae radix* Rhizoma, and *Agastachis* Herba. Typically, this herb is advised for COVID-19 patients who exhibit clinical signs of fever, fatigue, and gastrointestinal problems [9]. In the other research, in the Merangin area, one of the regencies in Jambi province Indonesia, the decoction of Sungkai leaves (*Peronema canescens*) (PC) has been used as one of the medicinal plants given to patients suffering from COVID-19. The local community believes that consuming a decoction of sungkai leaves in combination with conventional medicine can speed up the healing of patients with confirmed COVID-19 [10].

However, studies regarding the pharmacological properties of PC are still limited. There is a lack of the study reporting the mechanism of action of PC as an immunomodulator. A computer-assisted drug design method using molecular docking simulation is extensively used in drug discovery. Molecular docking simulation allows for homeostasis, finding new compounds of medicinal interest, predicting ligand-target molecular interactions, and defining structure activity [11]. The *in silico* approach quickly enables the filter of millions of compounds, thus reducing costs and increasing the probability of locating the targeted medication candidate. This study reports the mechanism of action of bioactive compounds from PC against IL-6 and TNF-α receptors based on binding energy, inhibition constant, and interactions with an amino acid. Bioactive compounds of PC can inhibit the expression of cytokines like IL-6 and TNF-α, which can be immunomodulatory in treating COVID-19 with an *in silico* approach.

MATERIALS AND METHODS

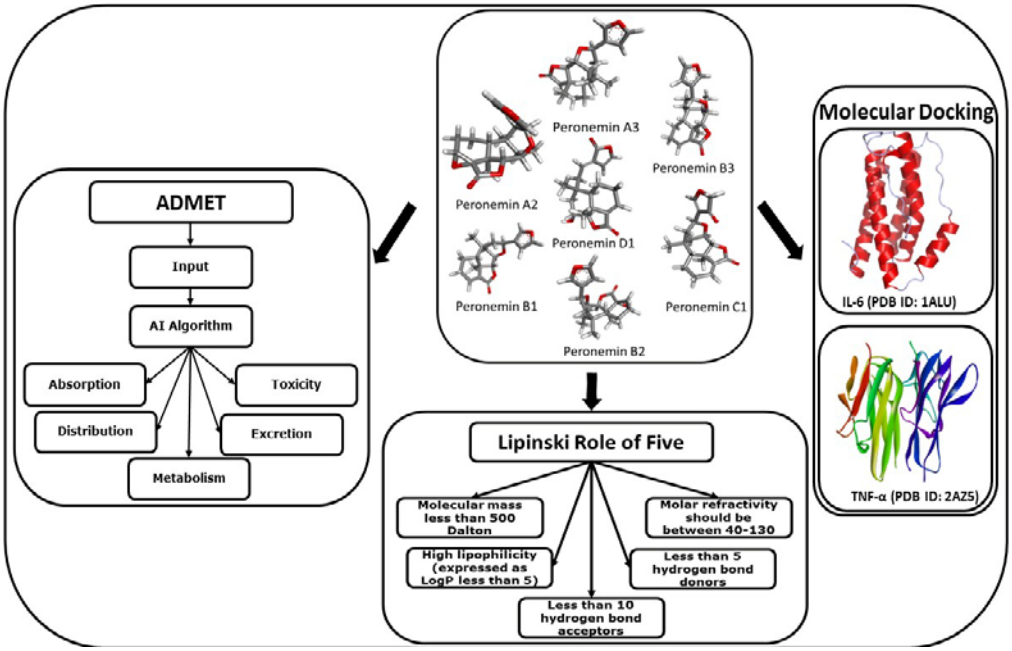


Fig. 1: Flow diagram showing *in silico* methods

Protein preparation

IL-6 is a protein crystal structure with (PDB ID: 1ALU), and TNF-α with (PDB ID: 2AZ5) was obtained from the protein databank (PDB) database [12]. Molecular docking simulation was executed and analyzed using AutoDock Tools software [13]. In molecular docking simulations, receptor proteins are prepared by removing ligands and water from active sites with Biovia Discovery Studio 2021 software [14]. Then, the receptor protein was added with polar hydrogen, charged with a Kollman atom, and torqued using the AutoDock Tools software [13]. The IL-6 receptor consists of a single chain containing an A chain, the active site outside the receptor. On the other hand, two chains, C and D, are utilized at the TNF-α receptor, and the active site is located between the C and D chains.

Ligand preparation

The ligands used in this study were bioactive compounds from PC [15]. Ligands were drawn in 2D structures using ChemDraw Professional 15.0 [16]. Geometry optimization with energy minimization using the MM2 method with Chem3D 15.0 [16]. The ligands were added with polar hydrogen, charged with Gasteiger, and torqued using the AutoDock Tools software [13].

Coordinates and grid box preparation

The coordinates were put based on the grid box's position. The grid box was created to cover the macromolecular residues responsible for the ligand's active binding. coordinates (-7,677;-12,743; 0.048) with the Grid Box size used in IL-6 is (40x40x40). While in TNF-α the coordinates used are (-19.163; 74.452; 33.837) with a Grid Box (40x40x40).

Molecular docking method validation

The molecular docking simulation was validated by re-docking the native ligand to the target. The native ligand of IL-6 obtained tartaric acid [17], and TNF-α obtained 6,7-dimethyl-3-[(methyl{2-[methyl({1-[3-(trifluoromethyl)phenyl]-1h-indol-3-Yl}methyl)amino]ethyl} amino)methyl]-4h-chromen-4-one [18]. In addition, using the overlay method, the molecular docking simulation technique was confirmed, the ligand between before and after molecular docking with Root Mean Square Deviation (RMSD) ≤ 2 [19].

Molecular docking

Simulation of molecular docking using the Autodock software [13] and the Lamarckian genetic algorithm (LGA) technique, maximum energy evaluation is 2.500.000, with 150 population sizes, the mutation is 0.02, crossover rates 0.80, and 100 running simulations [20]. Analysis of the results of molecular docking by looking at the lower value of constant of inhibition (Ki in nM), binding energy (ΔG in kcal/mol), and ligand interactions with amino acids [13].

Evaluation of drug likeliness, absorption, distribution, metabolism and excretion (ADME), and toxicity prediction

Evaluation of drug likeliness follows Lipinski's rule of five. Explanation of experimental and computational methods for assessing solubility and permeability in drug development and discovery. Lipinski's five rules predict poor absorption or penetration, which is more likely when there are molecular weight greater than 500, 10 H-bond acceptors, greater than 5 H-bond donors, and CLogP>5 (MlogP is over 4.15) [21]. Lipinski rules are analyzed employing the website http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp. Furthermore, the website pkCSM is used to predict the results of pharmacokinetic and toxicological (ADMET) [22]. ADMET predictions include absorption (Caco2 permeability), distribution (BBB permeability), metabolism (CYP2D6 substrate), excretion (total clearance), and toxicity (AMES toxicity).

RESULTS

Table 1: Molecular structures of diterpenoids of bioactive compound from PC

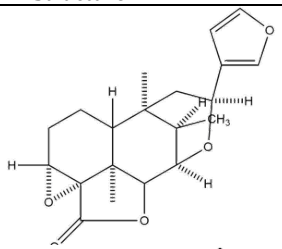
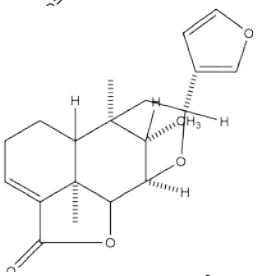
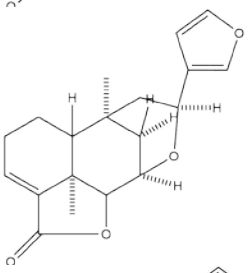
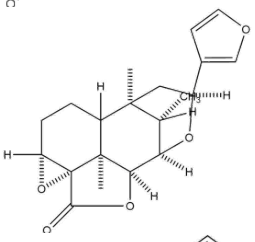
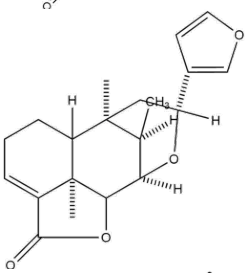
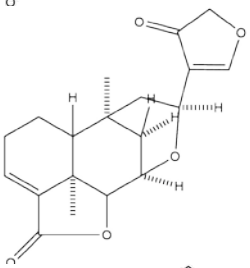
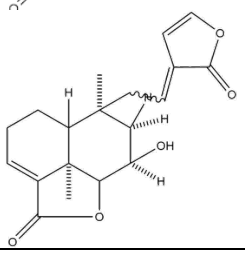
NO	Molecule name	2D Structure
	Peronemin A2	
2	Peronemin A3	
3	Peronemin B1	
4	Peronemin B2	
5	Peronemin B3	
6	Peronemin C1	
7	Peronemin D1	

Table 2: Molecular docking properties of bioactive compound from PC and native ligand by Autodock4

No	Molecule name	IL-6			TNF-α		
		ΔG in kcal/mol	Ki in nM	Interactive amino acids	ΔG in kcal/mol	Ki in nM	Interactive amino acids
1	Peronemin A2	-5.94	43.64	Arg 179, Arg 182, Arg 30, Leu 178	-8.82	343.59	Tyr 59, Tyr 119, Tyr 151
2	Peronemin A3	-6.30	24.02	Arg 179, Arg 182, Gln 175	-8.69	428.20	Tyr 151, Tyr 119, Gly 121, Leu 57
3	Peronemin B1	-6.60	24.02	Arg 182, Arg 179, Leu 178	-8.66	451.02	Gly 121, Tyr 119, Tyr 151, Tyr 59
4	Peronemin B2	-6.18	29.55	Arg 182, Arg 179, Leu 178	-7.89	1.65	Tyr 119, Tyr 59, Tyr 151
5	Peronemin B3	-6.60	14.61	Arg 182, Arg 179, Leu 178	-8.67	443.72	Gly 121, Tyr 151, Tyr 59, Tyr 119
6	Peronemin C1	-7.19	5.39	Arg 179, Arg 182, Gln 175	-8.86	320.42	Tyr 59, Tyr 119 Gly 121
7	Peronemin D1	-6.65	13.43	Arg 179, Arg 182, Gln 175	-3.35	3.48	Lys 11, Leu 55, Leu 157, Gly 54
8	Native Ligand	-5.91	46.27	Gln 175, Arg 179, Arg 182	-9.29	155.02	Gly 121, Tyr 59, Tyr 151, Tyr 119,

Table 3: Lipinski properties of bioactive compound from PC by scfbio online

No	Molecule name	Molecular weight	Log P	Hydrogen bond donors	Hydrogen bond acceptors	Molar refractivity
1	Peronemin A2	344	2.71	0	5	87.17
2	Peronemin A3	328	4.03	0	4	87.00
3	Peronemin B1	314	3.79	0	4	82.45
4	Peronemin B2	344	3.24	0	5	86.52
5	Peronemin B3	328	4.03	0	4	87.00
6	Peronemin C1	330	2.30	0	5	83.84
7	Peronemin D1	330	2.41	1	5	85.46

Table 4: ADMET properties of bioactive compound from PC by pkCMS online

No	Molecule name	Absorption (Caco2 permeability) (log Papp in 10-6 cm/s)	Distribution (BBB permeability) (log BB)	Metabolism (CYP2D6 substrate) (Yes/No)	Excretion (Total clearance) (log ml/min/kg)	Toxicity (AMES toxicity) (Yes/No)
1	Peronemin A2	1.12	0.74	No	0.47	No
2	Peronemin A3	1.36	0.66	No	0.89	No
3	Peronemin B1	1.36	0.66	No	0.89	No
4	Peronemin B2	1.12	0.74	No	0.47	No
5	Peronemin B3	1.36	0.66	No	0.89	No
6	Peronemin C1	1.32	0.24	No	0.96	No
7	Peronemin D1	1.05	-0.23	No	1.08	Yes

A B

Fig. 2: Three-dimensional crystal structure of the molecular target (A) IL-6 (B) TNF-α

A B

Fig. 3: Overlay conformation of the complex ligand. White (before molecular docking simulation), red (after molecular docking simulation). (A) Complex ligand IL-6 (B) Complex ligand TNF-α

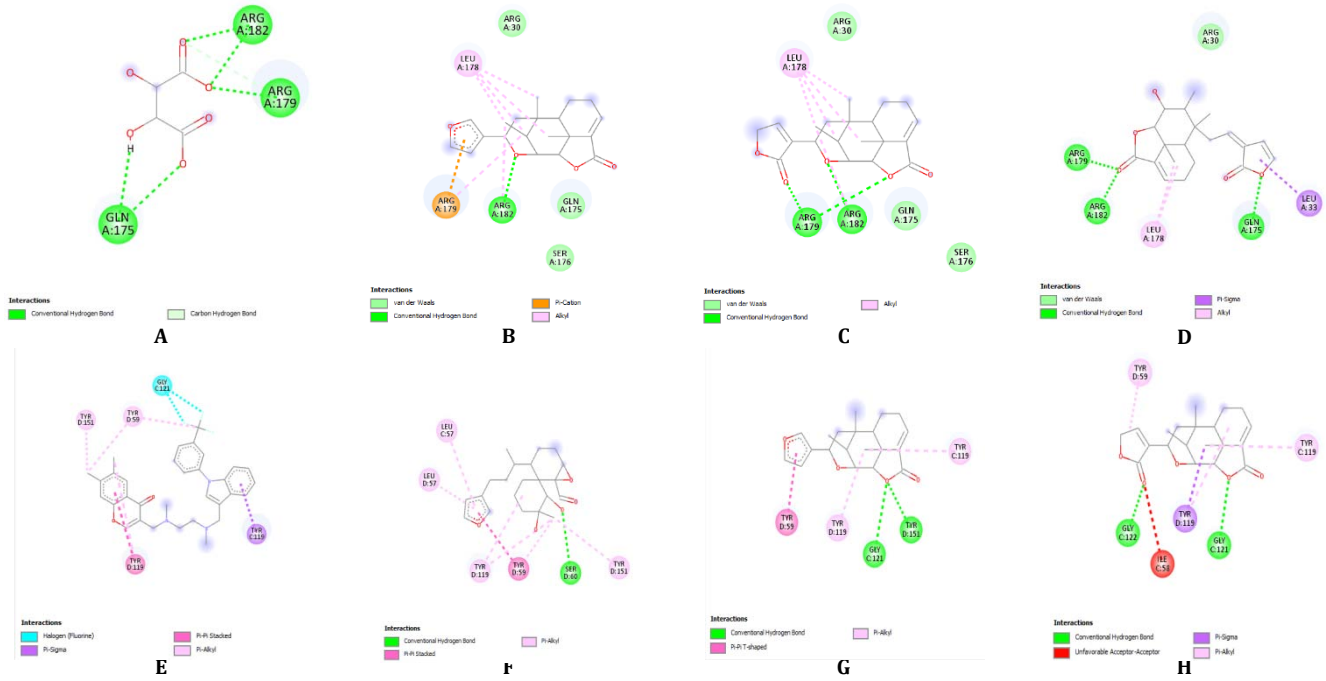


Fig. 4: 2D-Interaction of tartaric acid (A), peronemin B3 (B), peronemin C1 (C), and peronemin D1 (D) against IL-6. 2D-Interaction of 6,7-Dimethyl-3-[(Methyl{2-[Methyl({1-[3-(Trifluoromethyl) Phenyl]-1h-Indol-3-Yl} Methyl) Amino] Ethyl} Amino) Methyl]-4h-Chromen-4-One (E), peronemin A2 (F), peronemin B3 (G), and peronemin C1 (H) against TNF-α

DISCUSSION

Molecular docking simulation is commonly employed in drug development to identify new molecules of therapeutic interest, molecular level prediction of ligand-target interactions, and characterize structure-activity with computational methods [11] (fig. 1). This study discusses the mechanism of action of bioactive compounds from *Peronema canescens* (PC). PC can treat COVID-19 by blocking the expression of cytokines like IL-6 and TNF-α using an *in silico* approach.

The IL-6 receptor has one chain, with an active site outside the chain. While TNF-α has two chains, the active site is between chains A and B in the middle of the receptor (fig. 2). Molecular docking simulation was validated by redocking between the native ligand and receptor. Redocking the IL-6 (PDB ID: 1ALU) receptor with native ligands obtained RMSD values of 0.81 at run 88. The redocking results from this study have an RMSD greater than the research [12] is 0.45. Validity of the molecular docking approach is achieved when the RMSD value is ≤2.0 Å [23]. Meanwhile, the lowest binding affinity (ΔG) was-5.91 kcal/mol, and the inhibition constant (Ki) was 46.27 nM at run 51. This study has a ΔG that is better than the research [23], which is 5.65 kcal/mol, with (Ki) 72.72 nM. Redocking TNF-α with native ligands obtained RMSD 1.05 at run 4. The redocking results from this study have an RMSD greater than the research [12] is 0.61. The slightest ΔG is-9.29 kcal/mol, and the Ki is 155.02 nM at run 73. This study has a ΔG better than the research [12], which is-7.74 kcal/mol, with (Ki) 2.07 nM. The results of redocking with native ligand overlayed against the origin are shown in (fig. 3).

Molecular docking was carried out between bioactive compounds peronemins B1, B2, B3, A2, A3, C1, and D1 from PC (table 1) against IL-6 and TNF-α receptors using AutodockTools 4.2.6. From molecular docking results, several data were obtained, such as free energy of the bond (ΔG), inhibition constant (Ki), and interaction of amino acid from ligand and receptor. A compound has potential if it has a high affinity. Affinity is the capacity of a ligand (bioactive compound) to attach to a receptor. The number of hydrogen bonds influences the affinity of the ligand to the receptor, the free energy of the bond (ΔG), amino acid residues, and the constant of inhibition (Ki) (table 2). The more hydrogen bonds, the stronger the bonds formed (fig. 4). Therefore, the presence of hydrogen bonds in common becomes an essential factor with several hydrogen bonds. Suppose the hydrogen bond produced by the bioactive compound is the same as the bond formed between the natural ligand and the receptor. This indicates that the bioactive compound can inhibit the target protein's activity by replacing the natural ligand position [24].

All bioactive compounds from PC have smaller ΔG and Ki values than the native ligand at the IL-6 receptor. While the TNF-α receptor, all the bioactive compounds from PC had ΔG and Ki values greater than the native ligand (table 2). The free bond energy (ΔG) and the inhibition constant (Ki) are also parameters for the bond's quality. The lower ΔG, the more spontaneous the bonds formed. The inhibition constant (Ki) is inversely proportional to the torsional energy. The smaller the Ki, the higher the torsional energy and the more stable the bonds formed. Therefore, ΔG and Ki describe the spontaneity and stability of the bonds formed [25].

Bioactive compounds from PC obtained the three best bioactive compounds with the lowest ΔG and Ki against the IL-6 receptor were peronemin C1, peronemin D1, and peronemin B3. The ΔG and Ki of the bioactive compound were (-7.19 kcal/mol; 5.39 nM), (-6.65 kcal/mol; 13.43 nM), and (-6.60 kcal/mol; 14.61 nM), respectively. The results of this study have ΔG greater than the research [12]-8.61 kcal/mol. The results of this study have better results than the research [26], where the best binding energy value obtained is-5.3 kcal/mol. Nevertheless, it exceeds the research [27] ΔG is-7.7 kcal/mol. In other research [28] ΔG is-7.3 kcal/mol. Based on these findings, the ΔG value for IL-6 (PDB ID: 1ALU) was around 7 kcal/mol.

Based on molecular docking results, hydrogen bonds formed between natural ligands and IL-6 receptors Arg 179, Gln 175, and Arg 182. Peronemins that have similarities are Peronemin B3 (Arg 179, Arg 182, and Gln 175), Peronemin C1 (Arg 179, Arg 182, and Gln 175), and Peronemin D1 (Arg 179, Arg 182, and Gln 175). In addition, this study's interaction ligand and amino acid residues are similar to the study [26], which bind to essential amino acid Arg 179 [17].

Peronemins that have the lowest ΔG and Ki values at the TNF-α (PDB ID: 2AZ5) receptor are peronemin C1, peronemin A2, and peronemin A3 (-8.86 kcal/mol; 320.42 nM), (-8.82 kcal/mol; 343.59 nM), and (-

8.69 kcal/mol; 428.20 nM) respectively. The results of this study have ΔG greater than the research [12]-7.65, [26]-5.4, [29] −8.4, and [28] −8.7 kcal/mol. Other studies have ΔG lesser than the research [30]–9.1 kcal/mol. The ΔG and Ki values of native ligands are (-9.29 kcal/mol; 155.02 nM) (table 2 and fig. 4).

Interaction of amino acid residues with native ligands on TNF-α receptors is obtained (Gly 121, Tyr 59, Tyr 151, Tyr 119). Interaction of amino acid residues and peronemins have similarities with native ligands peronemin A2 (Tyr 59, Tyr 119, and Tyr 151), peronemin B3 (Tyr 59, Tyr 151, Tyr 119, and Gly 121), and peronemin C1 (Tyr 59, Tyr 119, and Gly 121) (fig. 4). The important interaction ligand and amino acid residue to the TNF-α receptor on the A and B chains is Tyr59, Tyr151, and Tyr119 [18]. Based on the research results, peronemins A2, B3, and C1 have the same interaction with amino acids as native ligands, namely Tyr59, Tyr151, and Tyr119. Except for the peronemins C1, which interact only with Tyr59 and Tyr119. The peronemins compound has activity against IL-6 and TNF-α because it has the same bond with a native ligand.

According to the data presented in (table 3), all bioactive chemicals from PC have a molecular weight of less than 500 mg/mol, the log P value, the value of donor and acceptor hydrogen bonds, and the value of molar refractivity conform to Lipinski's requirements. Lipinski's rule can determine the physicochemical properties of the bioactive compound (ligand) to determine a compound's hydrophobic/hydrophilic character in cell membranes through passive diffusion. The log P value represents The coefficient of fat/water solubility, which ranges from-0.4 to 5. Over 500 Da, molecules cannot diffuse across cell membranes. A molecule is increasingly hydrophobic as its log P value increases. Excessively hydrophobic molecules likely have a high toxicity level because they are maintained longer in the lipid membrane and disseminated more broadly throughout the body. As a result, the selectivity of their binding to the target enzyme is decreased. If the chemical cannot penetrate the lipid bilayer membrane, a log P value that is too negative is likewise undesirable. The greater the hydrogen bonding capability, the greater the absorption energy required [31].

Prediction of ADMET (table 4) is essential in assessing the pharmacokinetics of drug candidate molecules [22]. Caco-2's increased permeability will result in a predictive value>0,09. Caco-2 cells consist of human colorectal cancer epithelial cells. Caco-2 monolayer cells are often used as an *in vitro* model of human intestinal mucosa to estimate the absorption of oral medications. Prediction of a bioactive compound of PC, which has a value>1, proves that the bioactive compound has good absorption if used orally.

Compounds were considered to have a blood-brain barrier permeability if they had a logBB value>0.3. A molecule with a logBB<0.1 was inadequately dispersed in the brain. The blood-brain barrier (BBB) is a physiological barrier that restricts the entry of most substances from the blood to the brain. In addition to enhancing the efficacy of pharmacologically active medications, the ability of a drug to enter the brain is a crucial parameter for reducing side effects and toxicity and enhancing its efficacy *in vivo* animal models quantified blood-brain permeability as logBB, the logarithmic ratio of brain to plasma drug concentration. The distribution predictions of the peronemins, which have a value of>0.3, except for the peronemin D1, is-0.232. Proves that the peronemins can cross the BBB well. CYP2D6 is a predictor that will assess whether cytochrome P450 will likely metabolize a particular molecule. Multiple medications are metabolized by cytochrome P450. 2D6 and 3A4 are the two primary isoforms responsible for drug metabolism. Prediction of metabolism of the peronemins, all of which are not metabolized in CYP2D6. Total clearance (CLtot) is a predictor of excretion in logs (ml/min/kg). The primary components of drug clearance are renal clearance (renal excretion) and hepatic clearance (liver metabolism and biliary clearance). Bioavailability is important for determining steady-state dose levels and concentrations. Prediction of excretion from peronemins, all have a total clearance below 1, except for peronemins D1 1.08.

AMES toxicity is widely used to assess the potential for mutagenic compounds using bacteria. A positive result indicates that the compound is cancer-causing and mutagenic. However, all of the toxicity predictions of bioactive compounds of PC are not mutagenic, except that peronemins D1 is mutagenic.

CONCLUSION

The study finds that based on molecular docking, Lipinski role of five, and ADMET structure-based prediction results, a bioactive compound from PC met the candidate criteria for inhibitor IL-6 and TNF-α as immunomodulatory. Furthermore, the peronemin C1, bioactive compounds from PC, showed the best molecular docking simulation, based on binding affinity, inhibition constants, and interactions with amino acids, while also meeting Lipinski's role of five prediction results shows that bioactive compounds from PC have good adsorption so that they can be considered in the preparation of oral administration. Furthermore, in ADMET prediction results, bioactive compounds from PC are non-mutagenic, except peronemin D1. Hence the potential for further development of immunomodulatory.

ACKNOWLEDGMENT

This research was funded by literature and online research grants, namely Hibah Riset Data Pustaka dan Daring (RPDP) scheme 2022, from the Directorate of Research, and Community Services, Universitas Padjadjaran.

AUTHORS CONTRIBUTIONS

All authors made equal contributions.

CONFLICT OF INTERESTS

Declared none

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