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Anti-obesity Activity of Chlorophyll *a* from Spirulina (*Arthrospira platensis)* Through the Inhibition of Pancreatic Lipase Enzyme

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ABSTRACT

Obesity is considered a metabolic disease and a risk factor for other diseases. Spirulina (*Arthrospira platensis*) a blue-green microalgae belonging to the cyanobacterium group is rich in chlorophyll *a*. Spirulina extract has been shown to have pancreatic lipase inhibitory activity. This study aimed to determine the anti-obesity activity of diethyl ether fraction and chlorophyll a from Spirulina extract. Spirulina was extracted by maceration in methanol:acetone (7:3). The pigments including chlorophyll were separated from the extract by fractionation with diethyl ether. Chlorophyll *a* was isolated from the diethyl ether fraction by preparative thin layer chromatography (Prep-TLC). Chlorophyll *a* was identified by ultra violet-visible (UV-Vis), and fourier transform infra-red (FTIR) spectrophotometry. The anti-obesity activity of both the diethyl ether fraction, and chlorophyll a was evaluated by pancreatic lipase inhibition assay *in vitro*. The anti-obesity activity of chlorophyll a was further assessed *in silico* by molecular docking with human monoacylglycerol lipase enzyme (PDB ID: 5ZUN). Drug-likeness, and ADMET properties of chlorophyll a were also predicted. Results showed that, both the diethyl ether fraction, and chlorophyll a from Spirulina extract exhibited anti-obesity activity with IC_{50} values of 167.16 ± 1.08 µg/mL, and 23.47 ± 0.83 µg/mL, respectively. The anti-obesity activity of chlorophyll a was comparable to that of the positive control (orlistat) with IC_{50} of 20.53 ± 1.97 µg/mL. Chlorophyll *a* also exhibited potent anti-obesity activity *in silico*, with a binding affinity of -11.66 kcal/mol to 5ZUN protein. However, chlorophyll *a* did not meet the drug-likeness criteria, and was predicted to hepatotoxic and cardiotoxic.

Keywords: Anti-obesity, Chlorophyll *a*, Pancreatic Lipase, Spirulina.

Introduction

According to the Basic Health Research (RISKESDAS) report in 2018, there has been a continuous increase in obesity rates among adults over 18 years of age in Indonesia, increasing from 10.5% in 2007 to 14.8% in 2013, and reaching 21.8% in 2018.[1] Obesity was termed a priority health issue in Indonesia's 2020-2024 National Medium-Term Development Plan (RPJMN), which aimed to reduce obesity prevalence to 21.8% by the end of 2024. On a global scale, the World Obesity Federation predicted in 2015 that 2.7 billion adults would be overweight by 2025 if current dietary patterns persist - an increase of 35% from 2 billion adults in 2014.[2] Additionally, the World Health Organization data in 2016 indicated that 650 million adults (13% of the global adult population) were classified as obese, while the prevalence of overweight individuals stood at 39%.

Obesity should not be viewed merely as a result of an unhealthy lifestyle with associated health risks; rather, it should be recognized as a disease and a significant risk factor for other conditions.[3]

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Obesity is a major concern due to its strong link to premature mortality and the high incidence of degenerative diseases, including type II diabetes, cardiovascular disease, hypertension, hyperlipidemia, and certain types of cancer.[4] As a result, individuals who are overweight or obese often pursue various methods to reduce their weight, such as modifying their diet, exercising, taking weight loss or slimming medications, or even opting for plastic surgery. Many of the synthetic weight loss drugs on the market, such as orlistat, are commonly used in these efforts.[5]

Orlistat is recognized to be used in the long-term to inhibit the absorption of dietary fat.[5] Orlistat works by inhibiting fat absorption, altering the body's fat metabolism by blocking the action of the lipoprotein lipase enzyme that breaks down fat, so that the fat is excreted through the feces. Fat can be absorbed if it has been converted by lipase into fatty acids from food that are not hydrolyzed into free fatty acids and glycerol, thus, some fats are not absorbed by the intestine.[6] However, long-term use of synthetic drugs like orlistat can lead to several undesirable side effects, including nausea, vomiting, dry mouth, anorexia, constipation, insomnia, and neurological symptoms.[7]

An alternative therapy for obesity involves the use of herbal remedies, such as spirulina algae extract. Spirulina, a species of cyanobacteria, is a blue-green algae known for its rich organic nutrients. The most commonly used species in medicine are *Spirulina platensis*, *Spirulina maxima*, and *Spirulina jusijormzs*, all of which are considered nutritional plants.[8] In the fields of medicine and pharmacy, spirulina has been widely utilized as an ingredient in health supplements due to its comprehensive nutritional profile. Chemically, spirulina consists of water (27.8%), protein (5.4%), carbohydrates (33.3%), fats, and fiber. Additionally, spirulina contains enzymes, nucleic acids, amino acids, and vitamins (A, B, C, D, E, and K). It is also rich in macrominerals like nitrogen, oxygen, calcium, and selenium, and its micromineral content can be 10-20 times higher than that of land plants.[9] Furthermore, spirulina is rich in vitamin B complex, phycocyanin, gamma-linolenic

acid, protein, and *β*-carotene. Both phycocyanin and *β*-carotene have antioxidant properties, contributing to spirulina's health benefits.[10] Based on the foregoing, spirulina offers multiple benefits. In addition to its rich nutrient content, spirulina can aid in weight loss by inhibiting fat metabolism, thereby reducing fat accumulation and lowering the risk of obesity through inhibition of pancreatic lipase.

Materials and Methods

Chemicals/Reagents
Methanol (Merck), acetone (Merck), dietyl ether (Merck), crude porcine pancreatic lipase (PPL), p-nitrophenyl butyrate (p-NPB, Sigma-Aldrich, USA, 98% purity, analytical grade) was used for pancreatic anti-lipase activity, phosphate buffer (pH 7.2), DMSO, and orlistat standard.

Equipment
The tools used in this study include analytical balance (Shimadzsu, Japan), Whatman paper, UV-Vis spectrophotometer (Shimadzu 1700, Japan), rotary evaporator (Heidolph, Germany), silica gel GF 254 plates, UV 254 lamps, capillary tubes, chromatographic chambers, fillers, volume pipettes, micropipettes, volumetric flasks, vortexer, cuvettes, multimode readers (Sinergy HTX), 96-well flat bottom microplates (Biologix), multichannel micropipette (Sinergy), FT-IR (Agilent Technologies Cary 630, USA).

Software
Lipase enzyme with PDB ID: 5ZUN downloaded from Protein Data Bank (PDB, www.rcsb.org). Molecular docking tools, including Autodock Tool 1.5.6. Sep_17_14 and Biovia Discovery Studio V21.1.0.2.20298.

Sample collection and preparation
Spirulina (*Arthrospira platensis*) powder samples were obtained from the LIPI Marine Bio-Industry Center, North Lombok (-8.403518087616678, 116.08005960203926). Spirulina was harvested in September 2021. Identification was carried out by Evi Amelia Siahaan - a taxonomist at the Marine Bioindustry Center, LIPI, Indonesia with voucher number B-167/III/DI.02/9/2021. The Spirulina powder was kept in a plastic container that contained sterile sea water and temporarily stored in a coolbox.[11,12]

Extraction
Spirulina powder (50 g) was extracted by maceration in 200 mL of methanol:acetone mixture (7:3) at room temperature for 10 minutes.[13] The extract was concentrated using a rotary evaporator (Heidolph, Germany) at a temperature below 30°C. The concentrated extract was fractionated with diethyl ether.[14] The fractions were concentrated using nitrogen gas and stored in a dark place.

Identification of chlorophyll a
Chlorophyll *a* in the ether fraction was identified using Thin Layer Chromatography (TLC) with a silica gel GF_{254} stationary phase and a mobile phase consisting of n-hexane: acetone: ether (6:3:2).[15]

Chlorophyll a isolation
Chlorophyll a in the ether fraction was isolated using preparative thin layer chromatography with the eluent mixture of n-hexane: ether: acetone (6:3:2), then the bluish green fraction was collected.

Identification of chlorophyll a
Chlorophyll a was identified by measuring the absorption spectrum using a UV-Vis spectrophotometer (Shimadsu UV 1700, Japan).[16] The dried chlorophyll *a* isolate was dissolved in acetone (p.a). The absorption was measured at wavelength range of 350-800 nm. The spectrum of chlorophyll *a* that emerged was compared with the literature.

Identification of chlorophyll a with FTIR
The chlorophyll *a* isolate (0.5-1.5 mg) was put into a sample holder, and scanned using FTIR (Agilent Technologies Carry 630, USA). The results obtained were analyzed based on functional groups at certain wave numbers.[17]

Anti-obesity activity evaluation
In vitro pancreatic lipase inhibitory assay
Anti-obesity activity of the diethyl ether fraction and chlorophyll a isolated from spirulina extract was assessed *via* pancreatic lipase inhibitory assay *in vitro*. The assay was carried out in 96-well plates on an ELISA reader. The enzyme stock concentration was determined to be approximately 0.1 mg/mL for every 1 mg of solid PPL powder dissolved in 1 mL of buffer solution (a). Solutions of varying concentrations (50, 100, 150, 200, and 250 µg/mL) of the diethyl ether fraction was prepared (b). p-nitrophenyl butyrate (p-NPB) (0.5%) was dissolved in 1% DMSO (c), then diluted with 50 mM phosphate buffer (pH 7.2) to a concentration of 2.5 mM in 100 µL (d). Solutions (a), (b), and (d) were mixed and incubated at 37˚C for 10 min. Each sample was replicated three times. The same procedure was repeated for chlorophyll a at comcentrations of 2.5, 5, 10, 20, and 40 µg/mL. Orlistat (2.5, 5, 10, 20, and 40 µg/mL) was used as the positive control, while 1% DMSO was used as the negative control. One unit of activity is defined as the reaction rate that produces 1 µmol p-nitrophenyl butyrate at 37˚C. Inhibition of lipase activity is expressed as a percentage decrease in activity when PPL is incubated with the test compound.[18]

In Silico study of chlorophyll a
Molecular docking simulation
The binding interaction between chlorophyll *a* and the lipase enzyme was analyzed using *in silico* molecular docking study. Docking experiments were carried out using AutoDock software to explain the interaction between the lipase enzyme binding site and chlorophyll *a*. The crystal structure of human monoacylglycerol lipase with PDB ID: 5ZUN in complex with compound 3l - native ligand with PDB ID: 9JX [(4R)-1-(2'-chloro[1,1'-biphenyl]-3-yl)-4-[4-(1,3-thiazole-2-carbonyl)piperazin-1-yl]pyrrolidin-2-one, $C_{24}H_{23}ClN_4O_2S$] were downloaded from the protein data bank (PDB): https://doi.org/10.2210/pdb5ZUN/pdb, and saved in pdb file format. The 3D structures of the test ligand (chlorophyll a), and the positive control ligand (orlistat) were downloaded from the pubchem website: https://pubchem.ncbi.nlm.nih.gov/ with CID 12085802 for chlorophyll a , and CID 3034010 for orlistat, respectively. The ligand structures were optimized, and Gasteiger charges were added. The ligands were docked with the lipase protein using AutoDockTools 1.5.6. Redocking (docking validation) was performed using the native ligand (5ZUN). The docking method is said to be valid if the RMSD value is less than 2 Å. The smaller the RMSD value, the closer the docking pose of the test ligand is to the natural ligand (co-crystallized ligand).[19, 20]

Drug-likeness and ADMET predictions
The drug-likeness of the ligand (chlorophyll a) was assessed based on Lipinski's Rule of Five, which utilizes both experimental and computational approaches to evaluate solubility and permeability in drug discovery and development. The Lipinski's Rule of Five suggests that poor absorption and permeability are likely when the molecular weight exceeds 500, the number of hydrogen bond acceptors is greater than 10, the number of hydrogen bond donors exceeds 5, and the calculated log P (ClogP) is higher than 5 (or MlogP > 4.15). ADMET predictions encompass absorption (CaCO2 permeability), distribution (BBB permeability), metabolism (CYP2D6 substrate), excretion (total clearance), and toxicity (AMES toxicity), as well as hERG (human Ether-à-go-go)I/II inhibition.[21]

Statistical analysis
Results for pancreatic lipase inhibitory activity was presented as mean ± standard deviation (SD) of triplicate determinations. Data were analysed by one way analysis of variance (ANOVA). significant differences between means were established at p-value < 0.05.

Results and Discussion

Pigments identification with TLC

The initial identification of chlorophyll *a* was performed using Thin Layer Chromatography (TLC). The results of the TLC analysis on the diethyl ether fraction are shown in Figure 1. Based on the TLC results, the extract and fractions of spirulina contained chlorophyll *a*, indicated by the presence of bluish-green stains (spot no. 5, 6, and 7), with Rf values of 0.64, 0.60, and 0.58, respectively. It has been shown from previous studies that chlorophyll *a* exhibits a bluish-green colour with Rf of 0.57-0.60 in TLC analysis using hexane:ether:acetone (6:3:2) mixture as eluent.[15,22] Similar finding was obtained in the present study, although, there were 3 spots displaying a bluish-green colour, these three spots are the same compound, namely; chlorophyll *a* of the different epimers. Epimers are stereoisomeric compounds that differ in configuration only at one stereogenic center.

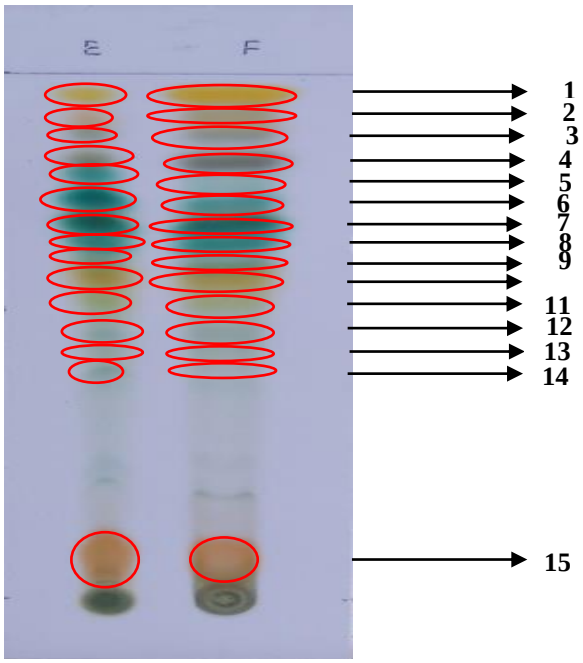


Figure 1: TLC chromatogram of extract (E) and diethyl ether fraction (F) of Spirulina extract. Stationary phase: silica gel GF_{254}, mobile phase: hexane:ether:acetone (6:3:2)

Isolation of chlorophyll a

Chlorophyll *a* was isolated from the pigment-rich diethyl ether fraction of Spirulina (*Arthrospira platensis*) extract using Preparative thin layer chromatography (Prep-TLC), chlorophyll *a* was isolated a yellowish-green band on the Prep-TLC chromatogram (Figure 2). From the Prep-TLC, 0.0172 g of chlorophyll *a* was obtained, corresponding to a yield of 0.13%. The chlorophyll content in spirulina has been found to be 10 times higher than that found in some types of land vegetables.[23]

Identification of Chlorophyll a

The UV-Vis spectrum and spectral data of chlorophyll *a* isolated from Spirulina is presented in Figure 3, and Table 1, respectively. The spectrum was measure across the visible wavelength range from 350 to 800 nm. The spectral pattern from this study was consistent with the spectral pattern of chlorophyll a in the literature, using the same solvent.[23]

From the spectral data, the wavelengths of maximum absorption (λmax) were recorded at 411 and 662 nm. These values are consistent with the maximum absorption wavelengths for chlorophyll *a* which has been reported to range from 400 to 430 nm, and at 662 nm.[24-27]

In addition to the UV-Vis spectroscopic analysis, the chlorophyll *a* isolate was also subjected to FTIR analysis, and the results are presented in Figure 4 and Table 2. Based on the FTIR spectral data, the following functional groups; $-CH_3$, -C=O, -CH, -CN, -OH, -C=C, and -C-O were identified in chlorophyll a.[28-30] These functional groups are consistent with the structure of chlorophyll *a* (Figure. 5).

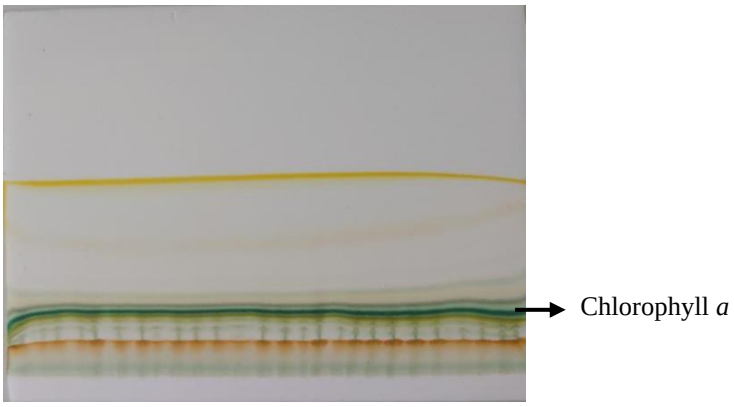


Figure 2: Preparative thin layer chromatography profile of chlorophyll a using silica gel GF_{254} as stationary phase and hexane:ether:acetone (6:3:2) as mobile phase

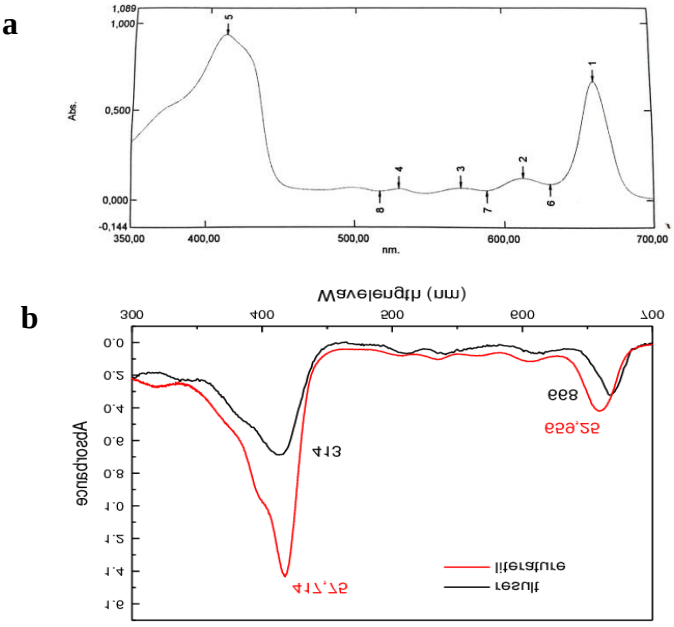


Figure 3: UV-Vis spectra of chlorophyll *a* before purification (a), and after purification with Preparative-TLC (b)

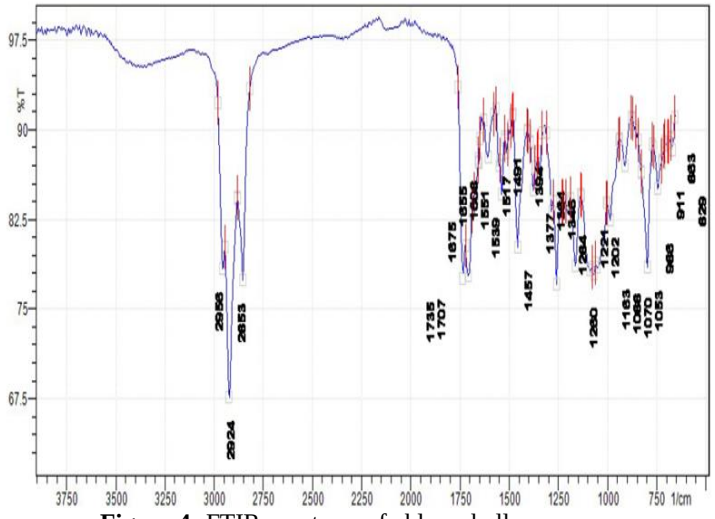


Figure 4: FTIR spectrum of chlorophyll *a*

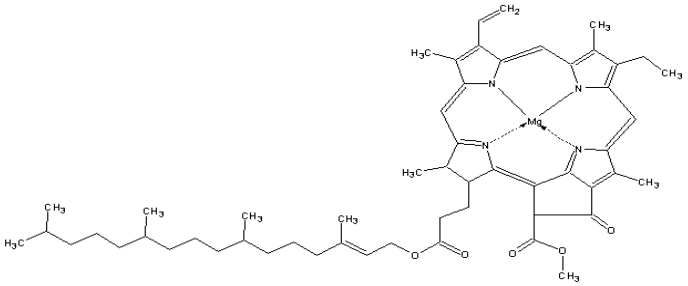


Figure 5: Structure of chlorophyll *a*

Table 1: UV-Vis spectral data of chlorophyll a from Spirulina

No	Maximum absorption wavelength (nm)	Absorbance
1	662	0.660
2	613	0.121
3	572	0.064
4	530	0.059
5	411	0.932

Table 2: FTIR spectral data of chlorophyll *a* from Spirulina

No	Type of bond	Vmax (cm^{-1})	Vmax (cm^{-1})[26,27,28]
1	CH_3	2924	2900
2	C=O	1675	1750
3	C=C	1606	1600
4	CH	2924 dan 2853	2925 dan 2872
5	CH3	1457	1440
6	CN	1053, 1070, 1088, 1183	1040, 1076, 1150
7	C=O	1675	1655 dan 1543
8	OH		3650-3200
9	C=C	1606	1700-1500
10	C-O	1053, 1070, 1088, 1163, 1202, 1221, 1264	1300-1000

Anti-obesity activity of chlorophyll a

In vitro pancreatic lipase inhibitory activity

The anti-obesity activity of the diethyl ether fraction and chlorophyll *a* isolated from Spirulina was assessed in terms of pancreatic lipase inhibitory activity, and the results are presented in Figure 6. Both the diethyl ether fraction, and the chlorophyll a, as well as the positive control (orlistat) exhibited a concentration-dependent increase in lipase enzyme inhibitory activity. The highest lipase inhibitory activity of 65.04 ± 1.72% was observed at 250 µg/mL for the diethyl ether fraction, while chlorophyll a exhibited highest activity of 69.29 ± 0.42% at a concentration of 40 µg/mL. The positive control (orlistat) on the other hand, exhibited 59.97 ± 2.00% lipase inhibition at the highest concentration of 40 µg/mL. Overall, chlorophyll a exhibited a higher lipase inhibitory activity (IC_{50} = 23.47 ± 0.83 µg/mL) than the diethyl ether fraction which gave IC_{50} value of 167.16 ± 1.08 µg/mL. The lipase inhibitory activity of chlorophyll a was comparable to that of the positive control (orlistat), which exhibited the highest lipase inhibitory activity, with IC_{50} value of 20.53 ± 1.97 µg/mL. These findings indicate that chlorophyll *a* in addition to phenolics, flavonoids, proanthocyanins, catechins, saponins, and triterpenoids, which have previously veen shown to possess pancreatic lipase inhibitory activity.[31] has the potential to be developed as natural treatment for obesity.

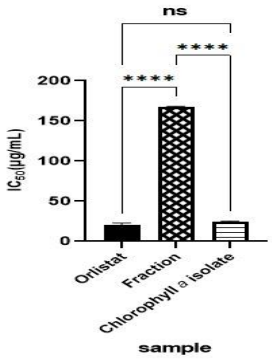


Figure 6: Pancreatic lipase inhibitory activity of diethyl ether fraction and chlorophyll a from Spirulina extract. ns = no significant, ****P < 0.0001

In silico pancreatic lipase inhibitory activity

The pancreatic lipase inhibitory activity of chlorophyll a was also tested *in silico* using the molecular docking method. As shown in Figure 7, docking pose or conformation of the natural ligand before and after the redocking or docking validation process were closely aligned, indicating minimal displacement. This was indicated by the low RMSD value, which was < 2 Å. This finding is consistent with previous study, which reported an RMSD value of 0.40 Å when some pharmacophore models were docked with 5ZUN protein.[32] The 5ZUN protein has main amino acid residues of Ser122, His269, and Asp239 in the active site.[33] Similarly, the oxyanion hole residues Gly50, Ala51, Met123, and Gly124 were identified at the ligand-binding site of the 5ZUN protein. The oxyanion hole serves as a crucial enzyme active site pocket, stabilizing the negative charge during the transition state that occurs with the deprotonation of oxygen or alkoxides. This structural feature is essential in stabilizing the intermediate reaction state during catalysis, particularly when the carbonyl oxygen carries a negative partial charge.[33] The 2D and 3D visualizations of the natural ligand's binding to the 5ZUN protein are shown in Figure. 8.

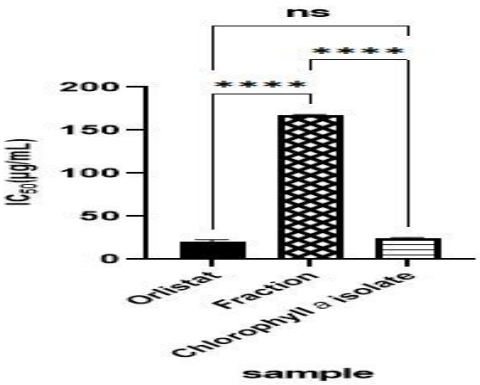


Figure 6: Pancreatic lipase inhibitory activity of diethyl ether fraction and chlorophyll a from Spirulina extract. ns = no significant, ****P < 0.0001

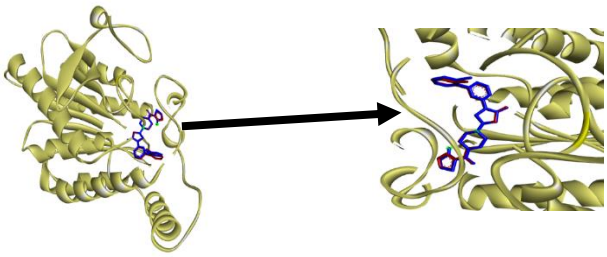


Figure 7: Overlay visualization of the 5ZUN complex with natural ligands before (red) and after (blue) the redocking process

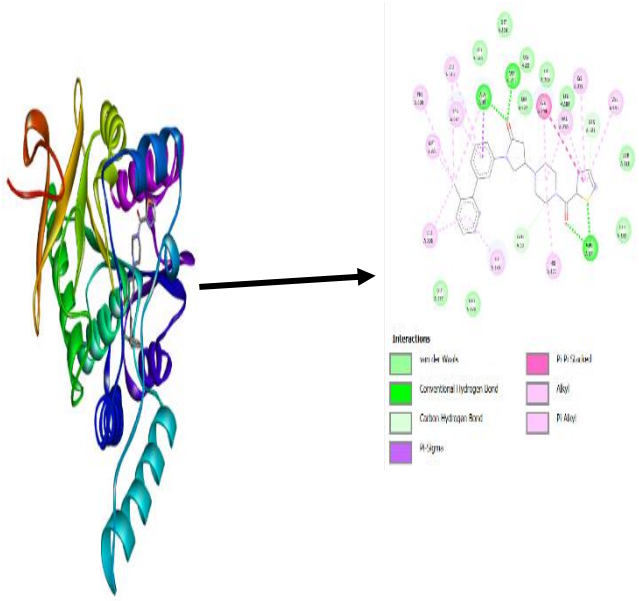


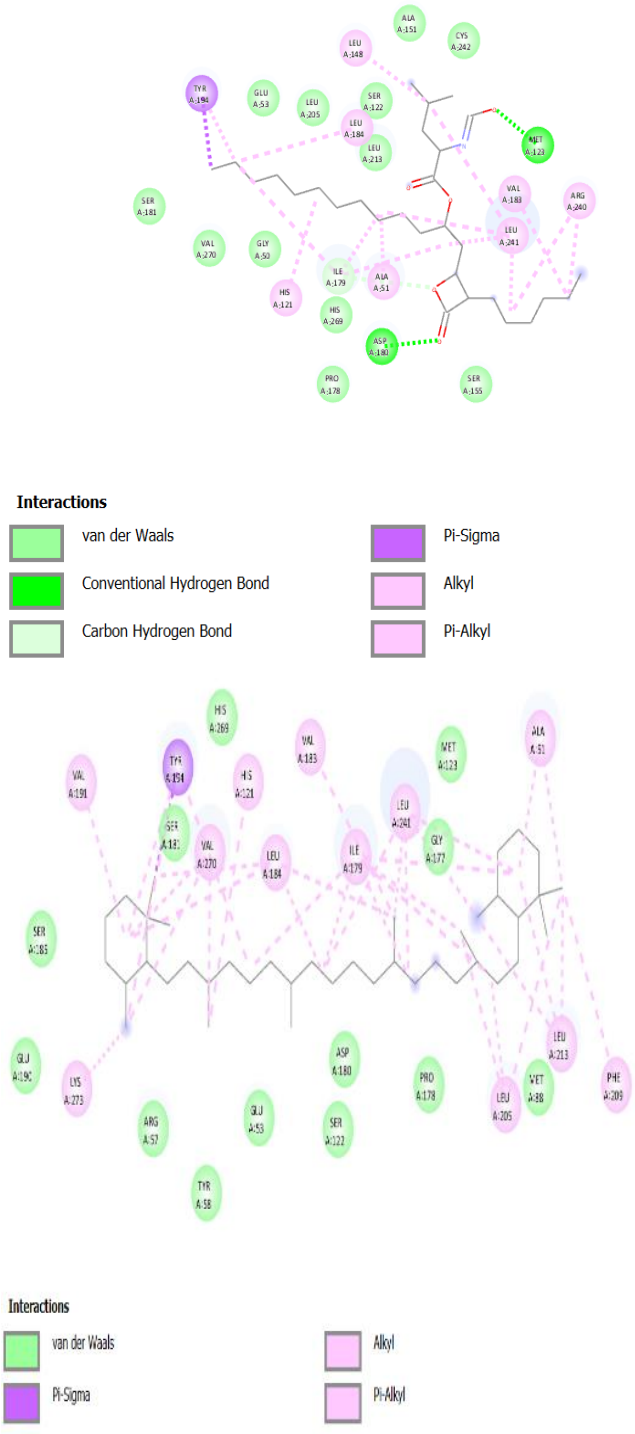
Figure 8: 3D and 2D visualizations of molecular docking results of 5ZUN protein with native ligand (4r)-1-(2'-chloro[1,1'-biphenyl]-3-yl)-4-[4-(1,3-thiazole-2carbonyl)piperazin-1-yl]pyrrolidin-2-one [c24h23cin4o2s]

Based on Figure 8, the interaction between the natural ligand and the amino acid residues Ser122, His269 at the protein active site, and Gly50, Ala51, and Met123 at the ligand binding site was evident. These interactions indicated the presence of oxyanion holes, which contributed to stabilizing the anionic transition state.[34] Analysis of the interactions between the ligands and the amino acid residues of the receptors or target proteins are presented in Table 3 and Figure 9.

Table 3 illustrates the interactions between the ligands and the target protein at amino acid residues marked with blue blocks in Figure 9. The interaction results further highlighted the presence of oxyanion holes, indicated by the yellow blocks in Figure 9, which aid in stabilizing the anionic transition state. Notably, the ligands demonstrated binding with Ser122, influencing the catalytic activity of the enzyme. In terms of binding affinity, chlorophyll a exhibit superior binding compared to orlistat. For a clearer visualization of the interactions between the ligands and the 5ZUN protein, a 2D image was presented (Figure 9).

Amino acids that play a role in the docking between the positive control compound orlistat and pancreatic lipase were used in assessing the similarity of binding (binding site similarity) of the docking results between test ligand and the reference ligand with the target protein as a measure of their ability to act as pancreatic lipase inhibitors.[33] Orlistat was used as a control for the *in silico* pancreatic lipase inhibitory assay because it is a known anti-obesity drug that works by inhibiting fat absorption through the inhibition of pancreatic lipase enzyme.

Based on the results shown in Table 3 and Figure 9, it can be seen that the amino acid residues involved in the binding of chlorophyll *a* with the receptor have many similarities with that of orlistat. However, Ser 122 amino acid residue in orlistat was bound with hydrogen bond so that the bond between orlistat and 5ZUN protein was stronger than that of chlorophyll *a* which had van der Waals bond at Ser 122 amino acid residue of 5ZUN protein. The number of hydrogen bonds affect the binding affinity of test ligands, from Table 3 and Figure 9, it was observed that the more the number of hydrogen bonds, the smaller the binding affinity. This may be due to the influence of other types of bonds, including van der Waals, covalent, sigma, and pi alkyl bonds which also affect binding affinity.



A B

Figure 9: 2D Visualization of molecular docking results of orlistat (A), and chlorophyll *a* (B) on lipase receptors (PDB ID: 5ZUN)

Table 3: Molecular docking interaction of test ligands with 5ZUN protein

No	Ligand	Binding affinities (kcal/mol)	Inhibition constant/Ki (μM)	Number of Hydrogen Bonds	Amino acid residue interactions
1	Orlistat	-6.67	12.93	3	Gly 50, Ala 51, Glu 53, Tyr 58, Met 88, His 121, Ser 122, Met 123, Leu 148, Ala 151, Gly 177, Pro 178, Ile 179, Asp 180, Leu 184, Tyr 194, Leu 205, Phe 209, Leu 213, Leu 241, His 269, Val 270
2	Chlorophyll *a*	-11.66	2.85 nM	0	Ala 51, Glu 53, Arg 57, Tyr 58, Met 88, His 121, Ser 122, Met 123, Gly 177, Pro 178, Ile 179, Asp 180, Ser 181, Val 183, Leu 184, Ser 185, Glu 190, Val 191, Tyr 194, Leu 205, Phe 209, Leu 213, Leu 241, His 269, Val 270, Lys 273

Note: Ser 122 and His 269: Interaction of ligand and target protein at amino acid residues
Gly 50, Ala 51 and Met 123: The interaction results show the presence of oxyanion holes.

Drug-likeness of chlorophyll a

The results of the drug-likeness prediction of chlorophyll a according to Lipinski's rule of five are presented in Table 4. Lipinski's Rule of Five, also known as Pfizer's Rule of Five, comprises a set of guidelines utilized in drug design to assess the likelihood of compounds achieving good oral bioavailability. Formulated by Christopher A. Lipinski in 1997, these rules are based on the observation that most orally administered drugs conform to specific criteria. They outline molecular properties essential for drug pharmacokinetics in the body, including absorption, distribution, metabolism, and excretion (ADME). According to Lipinski's rule of five, an orally active drug should not exceed one violation of the following criteria: no more than 5 hydrogen bond donors (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds), no more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms), a molecular mass of less than 500 daltons, an octanol-water partition coefficient (log-P) that does not exceed 5, and a polar surface area of 40-140 $Å^2$.[35, 36]

Table 4: Pharmacokinetic (ADMET) profile of chlorophyll *a*

Pharmacokinetic Parameters	Model Name	Prediction Chlorophyll *a*	Unit
Absorption	Water solubility	-4.272	log mol/L
	CaCO-2 Permeability	0.676	log Papp in 10^{-6} cm/s
	Intestinal absorption (human)	100	% Absorbed
	Skin Permeabitity	-2,735	log Kp
	P-glycoprotein substrate	*No*	*Yes/No*
	P-glycoprotein I inhibitor	*Yes*	*Yes/No*
	P-glycoprotein II inhibitor	*Yes*	*Yes/No*
Distribution	VDss (human)	0.164	log L/kg
	Fraction unbond (human)	0.092	Fu
	BBB permeability	-1.621	Log BB
	CNS permeability	-2.129	Log PS
Metabolism	CYP2D6 substrate	*No*	*Yes/No*
	CYP3A4 substrate	*Yes*	*Yes/No*
	CYP2C19 inhibitor	*No*	*Yes/No*
	CYP2C9 inhibitor	*No*	*Yes/No*
	CYP2D6 inhibitor	*No*	*Yes/No*
	CYP3A4 inhibitor	*Yes*	*Yes/No*
Excretion	Total Clearance	-0.695	log ml/min/kg
Toxicity	AMES toxicity	*No*	*Yes/No*
	Max. Tolerated dose (human)	0.306	Log mg/kg/day
	hERG I inhibitor	*No*	*Yes/No*
	hERG II inhibitor	*Yes*	*Yes/No*
	Oral Rat Acute Toxicity (LD50)	2.324	Mol/kg

Oral Rat ChronicToxicity (LOAEL)	0.047	log mg/kg_bw/day	
Hepatotoxicity	*Yes*	*Yes/No*	
Skin Sensitisation	*No*	*Yes/No*	

Based on the data presented in Table 4, the test ligand (chlorophyll a) has a molecular weight of 893.509 daltons, which exceeds the recommended limit of 500 daltons. The log P was 12.64946, significantly higher than the maximum allowable of 5. The number of hydrogen bond donors was 20, exceeding the limit of 5, while the number of hydrogen bond acceptors was 7, which is within the acceptable range of 10. The polar surface area was 403.490 Å², also exceeding the upper limit of 140 Å². From these data, it can be concluded that only one of the parameters met the requirements, therefore, chlorophyll a is not recommended as an orally active drug.

Molecular weight will determine the permeability of an active compound. In addition, higher hydrogen bond acceptor and donor values indicate poor permeability of active compounds, this will affect the absorption and distribution process of an active compound.[36, 37] Log P is a measure of the relative solubility of a compound in octanol and water, and its value affects the physicochemical and pharmacokinetic properties of a compound. Compounds with high log P tend to be more lipophilic, which can affect the absorption, distribution, and availability of drugs in the body. Therefore, Log P is one of the important parameters analyzed in the context of a compound's potential to become a new drug according to Lipinski's rule. Log P value of < 5 is set as a limit to predict the nature of active compounds that tend to be hydrophilic, this indicates that the compound is more soluble and has high permeability.[21] The higher the log P value for a drug compound, the higher the likelihood of toxicity, because the drug will be retained longer in the lipid bilayer and will be distributed more widely in the body so that the selectivity of binding to target proteins is reduced.

Polar surface area (PSA) is one of the five parameters of Lipinski's rule of five used to assess the potential of a compound to become a new drug. According to this rule, a compound will have high absorption or permeability if the PSA value is less than 140 Å². This rule helps in distinguishing between molecules that have drug potential and those that do not. Polar surface area is one of the important aspects in the physicochemical and pharmacokinetic prediction of a compound, and its value must meet the criteria of Lipinski's rule for the test compound to become a new drug.[38]

In Lipinski's Rule, hydrogen bond donors and hydrogen bond acceptors refer to the maximum allowable numbers of these interactions in a compound being evaluated for potential new drug. Hydrogen bond donors are defined as the total number of nitrogen-hydrogen and oxygen-hydrogen bonds, while hydrogen bond acceptors are the sum of all nitrogen and oxygen atoms present in the compound.[34] If a compound fails to meet the minimum criteria for hydrogen bond donors, this may indicate its potential for poor absorption and permeability. Compounds with more than five hydrogen bond donors tend to exhibit an increased capacity to form hydrogen bonds, which can negatively impact absorption. Similarly, compounds with more than ten hydrogen bond acceptors may also experience compromised permeability.[39]

ADMET predictions revealed chlorophyll a to be potentially hepatoxic, and exhibited cardiotoxicity by inhibition of hERG II gene (Table 4). The human ether-a-go-go related gene (hERG) encodes a tetrameric potassium channel, which plays crucial role in cardiac action potential. Inhibition of hERG channel may result in long QT syndrome (LQTS), and cause avoidable sudden cardiac death. Hence, hERG inhibition is a useful predictor of cardiotoxicity of a test compound.[40,41]

Conclusion

The findings from the present study have shown that the pigment-rich diethyl ether fraction of Spirulina extract as well as chlorophyll a isolated thereof have anti-obesity activity by inhibiting the activity of pancreatic lipase *in vitro*. Chlorophyll a exhibited higher pancreatic lipase inhibitory activity than the diethyl ether fraction, and this activity was comparable to that of the positive control (orlistat). Chlorophyll *a* also exhibited potent anti-obesity activity *in silico*, with a binding affinity of -11.66 kcal/mol, which was stronger than that of orlistst (-6.67 kcal/mol). Chlorophyll a bound to key amino acid residue Ser122 in the enzyme (5ZUN) active site. However, chlorophyll *a* did not meet the Lipinsky's rule of five requirements and was predicted to be hepatotoxic, and cardiotoxic by acting as hERG II inhibitor.

Conflicts of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that anyliability for claims related to the content of this article shall be borne by them.

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References

1. Ministry of Health. Riskesdas RI. 2018; 53(9):1689–1699.
2. Heber D and Li Z. Primary Care Nutrition: Writing the Nutrition Prescription. 1st ed., CRC Press. 2017. DOI: 10.1201/9781315152165.
3. Sudargo T, LM HF, Rosiyani F, Kusmayanti NA. Diet and Obesity . UGM Press, Yogyakarta; 2018.
4. Tadesse Y, Derso T, Alene KA, Wassie MM. Prevalence and Factors Associated with Overweight and Obesity Among Private Kindergarten School Children in Bahirdar Town, Northwest Ethiopia: Cross-Sectional Study. BMC Res Notes. 2017; 10(22):1–6. https://doi.org/10.1186/s13104-016-2308-8
5. Hamdy O. Orlistat. Retrieved Januari 25, 2016; from emedicine: http://www.emedicine.medscape.com/article/123702-overview.
6. Tjay TH and Rahardja K. Essential Medicines. PT. Elex Media komputindo, Jakarta; 2015.
7. Samout N, Ettaya A, Bouzenna H, Ncib S, Elfeki A, Hfaiedh N. Beneficial Effects of Plantago albicans on High-Fat Diet-Induced Obesity in Rats. Biomed Pharmacother. 2016; 84:1768–1775. doi: 10.1016/j.biopha.2016.10.105.
8. Setia A, Sutiyarso, Darwis I. Effect of Spirulina Extract (Arthrospira) Administration in Obesity Therapy. J Medula. 2019; 9(2):398–402. https://doi.org/10.53089/medula.v9i2.261
9. Park HJ and Lee HS. The influence of obesity on the effects of spirulina supplementation in the human metabolic response of Korean elderly. Nutr Res Pract. 2016; 10(4):418–423. https://doi.org/10.4162/nrp.2016.10.4.418
10. Capelli B and Cysewski GR. Potential Health Benefits of Spirulina Microalgae. Nutrafoods. 2010; 9:19–26. https://doi.org/10.1007/BF03223332
11. Nugraheni SA, Khoeri MM, Sabdono A, Radjasa OK. Antibacterial Activity Of Bacterial Symbionts Of Softcoral *Sinularia* sp . Against Pathogenic Resistant Bacteria. J Coast Dev. 2010; 13(2):113–118
12. Sabdaningsih A, Cristianawati O, Sibero MT, Nuryadi H, Radjasa OK, Sabdono A, Trianto A. Screening Antibacterial Agent from Crude Extract of Marine-Derived Fungi Associated with Soft Corals against *MDR-Staphylococcus*

haemolyticus. IOP Conf Ser: Earth Environ Sci. 2017; 55(1):012026. DOI 10.1088/1755-1315/55/1/012026
13. Sulistiyani Y, Sabdono A, Afiati N, Haeruddin. Fucoxanthin Identification and Purification of Brown Algae Commonly Found in Lombok Island, Indonesia. Biodiversitas. 2021; 22(3):1527-1534. https://doi.org/10.13057/biodiv/d220358
14. Kusmita L, Puspitaningrum I, Limantara L. Identification, Isolation and Antioxidant Activity of Pheophytin from Green Tea (*Camellia Sinensis* L. Kuntze). Procedia Chemistry. 2015; 14:232-238. https://doi.org/10.1016/j.proche.2015.03.033.
15. Wang BJ, Yu Z, Hwang LS. Quantitative Analyses of Chlorophylls and Their Derivatives by Thin Layer Chromatography. J of the Chinese Agr Chem Soc. 1995; 33:550–560. https://doi.org/10.1016/0005-2728(68)90109-6
16. Mahendra E, Kusmita L, Susanto A, Limantara L. Deaggregation of Crude Pigment Extract (*Sargassum crasifolium*) J.G. Agardh In Acetone and Methanol Solvents. Akuatik: J Sumberdaya Perairan. 2008; 4:29–36.
17. Riwanti P, Andayani R, Trinanda L. Antibacterial Activity Test of *Sargassum polycystum* against *Staphylococcus aureus* Bacteria. J Pharm Sci. 2021; 6:19–23.
18. Liu T-T, Liu,X-T, Chen Q-X, Shi Y. Lipase Inhibitors for Obesity : A Review. Biomed Pharmacother. 2020; 128:110314 https://doi.org/10.1016/j.biopha.2020.110314
25. Rachma D, Sandiningtyas V, Suendo V. Isolation of chlorophyll a from spinach and its modification using Fe^{2+} in photostability study. Proceedings of the Third International Conference on Mathematics and Natural Sciences. 2010.
26. Gross J. Pigments In Vegetables - Chlorophylls And Carotenoids, Springer Science Business Media, LLC. 1991.
27. Munawaroh HSH, Fathur RM, Gumilar G, Aisyah S, Yuliani G, Mudzakir A. Characterization and Physicochemical Properties of Chlorophyll Extract from *Spirulina* sp. J Phys: Conf Ser. 2019; 1280:022013. DOI 10.1088/1742-6596/1280/2/022013
28. Manrich A, Oliveira JE, Martins MA, Mattoso LHC. Physicochemical and Thermal Characterization of the Spirulina platensis. J Agric Sci Technol B. 2020; 10:298–307. DOI:10.17265/2161-6264/2020.05.004
29. Rifa'i A, Hidayah H, Narudi, Ibrahim AM. The Role of Chlorophyll Extract from Ambarella Leaves (*Spondias dulcis* Forst) in Dye Sensitized Solar Cells. J Itekima. 2019; 6:24–34.
30. Coates J. Interpretation of infrared spectra, a practical approach. EAC. 2000; 12:10815-10837. https://doi.org/10.1002/9780470027318.a5606
31. Garza ALD, Milagro FI, Boque N, Campión J, Martínez JA. Natural Inhibitors of Pancreatic Lipase as New Players in Obesity Treatment. Planta Med. 2011; 77(8):773–785. DOI: 10.1055/s-0030-1270924
32. Jha V, Biagi M, Spinelli V, Granchi MDSC, Poli G, Tuccinardi T. Discovery of Monoacylglycerol Lipase (MAGL) Inhibitors Based on a Pharmacophore-Guided Virtual Screening Study. Molecules. 2021; 26(1):78. https://doi.org/10.3390/molecules26010078
33. Kashyap A, Rani D, Kumar S, Bhatt S. Design and Computational Evaluation of New Carbamate Derivatives for the Inhibition of Monoacylglycerol Lipase Enzyme By Using Docking. Int J Pharm Sci Drug Res. 2023; 15(5):665–674. https://doi.org/10.25004/IJPSDR.2023.150515.
34. Berdan CA, Erion KA, Burritt NE, Corkey BE, Deeney JT. Inhibition of monoacylglycerol lipase activity decreases glucose-stimulated insulin secretion in INS-1 (832/13) cells and rat islets. PLoS ONE. 2016; 11(2):1–14. https://doi.org/10.1371/journal.pone.0149008
35. Nusantoro YR and Fadlan A. Drug-Like Properties Analysis, ADMET Prediction, and Molecular Docking of Isatinyl-2-Aminobenzoylhydrazone and Transition Metal Complexes Co(II), Ni(II), Cu(II), Zn(II) Against BCL2-XL. Akta Kimia Indones. 2020; 5(2):114. DOI: http://dx.doi.org/10.12962/j25493736.v5i2.7881
36. Odoemelam CS, Hunter E, Simms J, Ahmad Z, Chang M-W, Percival B. Williams IH, Molinari M, Kamerlin SCL, Wilson PB. *In Silico* Ligand Docking Approaches to Characterise the Binding of Known Allosteric Modulators to the Glucagon-Like Peptide 1 Receptor and Prediction of ADME/Tox Properties. Appl Biosci. 2022; 1(2):143–162. https://doi.org/10.3390/applbiosci1020010
37. Vastiani F, Lestari RD, Damayanti DS. Antilipemic Potential of Active Compounds of Kombucha Soursop Leaves (*Annona Muricata* Linn.) Against PPAR-α and Lipoprotein Lipase Target Proteins with *In Silico* Studies. J Kedokteran Komunitas. 2022; 10:1–10.
38. Hartono H, Fadli Z, Bintari YB. *In Silico* Test: Antibacterial Activity of Active Compounds of *Gracilaria verrucosa* Against *Staphylococcus aureus*. J Bio Komplementer Med. 2022. 9:1–12. DOI: https://doi.org/10.26740/lenterabio.v13n1.p44-54
39. Maftucha N, Manalu R, Amelia R, Cordia P, Bupu R. Potential of Xanthone Derivative Compounds from Mangosteen Peel (*Garcinia mangostana* L.) as *Mycobacterium tuberculosis* Protein Inhibitors: *In Silico* Study. Pharm J Indones. 2022; 7(2):123–128. DOI: https://doi.org/10.21776/ub.pji.2022.007.02.7
40. Sanguinetti MC and Tristani-Firouzi M. hERG potassium channels and cardiac arrhythmia. Nature. 2006; 440:463–469.
41. Wang S, Li Y, Xu L, Li D, Hou T. Recent developments in computational prediction of HERG blockage. Curr Top Med Chem. 2013; 13:1317–1326.