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In Silico Compounds Analysis in Ginger (Zingiber Officinale) as Candidate for Acute Coronary Syndrome Medication

James Ibrahim[1], Dian Ratih Laksmitawati[2]*, Esti Mulatsari[3], Esti Mumpuni[4]
Faculty of Pharmacy, Pancasila University, South Jakarta
Corresponding Author: Dian Ratih Laksmitawati
dian.ratih@univpancasila.ac.id

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A B S T R A C T

Based on the World Health Organization and other authorized health organizations, heart disease is one of the diseases with the largest contributor to death in the world, including Acute Coronary Syndrome with an estimated number of patients of 126 million in 2020. This research entails phytoconstituents of *Zingiber officinale* as possible Acute Coronary Syndrome medication with *in silico* methods by altering the activity of HMG-CoA Reductase, PPAR-α, PPAR-γ, NPC1L1, β_1-AR, ACE and $P_2Y_{12}R$. This research used Molegro Virtual Docker 6.0 as tool to analyze the compounds with docking computation approach and also utilize Protein Data Bank (PDB) files : 1HWK, 2ZNN, 4EMA, 4AMJ, 2YDM, 4PXZ and 7DFZ. Chemdraw 3D was used in order to minimize the ligand's energy. Furthermore, to analyze the pharmacokinetics aspects, this study involved pkCSM and SwissADME to predict a few parameters in each aspect, and finalized the research with Dynamic Molecular approach with YASARA.

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INTRODUCTION

Cardiovascular is one of the fundamental parts of the human body systems due to its role in circulating blood containing oxygen and nutrients to all organs and tissues. This system cannot be separated from the role of the heart as the main organ and vascular as a supporting part. With age, the condition of the cardiovascular system can decline and experience various problems such as valve disorders, cardiomyopathy, and the most common one is Acute Coronary Syndrome. As the name suggests, Acute Coronary Syndrome is a collection of symptoms such as chest pain, heart-locked feeling, and extreme pain in the jaw, and upper arm that refer to a reduction or decrease in the supply of blood flow to the heart. Although Acute Coronary Syndrome (ACS) and Coronary Artery Disease (CAD) are often used interchangeably, they describe different conditions. Basically, CAD is a condition that can be characterized by atherosclerotic and can be present without any symptoms while ACS also can be defined as atherosclerotic condition but presents with multiple symptoms and strongly associated with Myocardial Infarction. ACS can also be divided into 3 subclasses namely STEMI (ST-Elevation Myocardial Infarction), NSTEMI (Non-ST Elevation Myocardial Infarction) and Unstable Angina. Although this disease has many names, all of them have the same bottom line, which is atherosclerosis as the main cause.

Atherosclerosis is a condition of narrowing of the arteries due to the accumulation of saturated fat, triglycerides and cholesterol in the form of LDL. LDL itself has properties that are detrimental to humans including its ability to stick to the walls of blood vessels and easily oxidized to become free radicals. LDL oxidation will cause inflammation in the arterial vessels and will further be responded by the immune system as a threat. Macrophages as the first line in the immune system will respond by trying to eliminate LDL stacks via FAT/CD36 (Fatty Acid Translocase) receptors which will further be processed by ABCA1R and ABCG1R (ATP Binding Cassette Receptor Type) to be converted into HDL. However, as the concentration of LDL increases which exceeds the capacity of macrophages, the FAT/CD36 receptors become saturated and are unable to operate optimally. As a result, LDL that cannot be neutralized by macrophages will develop into foam cells and trigger the activation of immune-related protein such as interleukin and chemokines and eventually will activate the calcium deposition in order to form a solid structure known as thrombus, causing rigidity in the blood vessels.

Acute Coronary Syndrome can also cause several complications that arise after myocardial infarction. There are at least the 3 most basic complications that patients may experience, such as decreased cardiac contractility due to slow regeneration in damaged heart muscle cells, cardiac electro gradient disorders can also occur if Acute Coronary Syndrome generate in the right atrium or ventricular and necrosis in tissue where damaged heart muscle cells will be responded by the body as a threat and try to eliminate it with necrosis. Prolonged necrosis can cause inflammation and will lead to pericarditis or other heart-inflammatory state.

A polypharmacy state develops as a result of the multiple drugs used to treat Acute Coronary Syndrome, which typically affects elderly people. These medications include statins, beta-blockers, vasodilators, fibrates, and others. While these drugs have been shown to significantly slow the progression of the disease, the polypharmacy itself can cause new problems for the patient, such as drug interactions due to CYP450 activity changes, an increase in the risk of adverse side effects, and a decrease in compliance in taking medicine due to memory loss with age, resulting in suboptimal drug action. The considerable increase in cases and mortality rate over time also suggests that existing techniques of treatment, whether invasive (Angioplasty and CABG) or non-invasive (oral medication), do not have a meaningful influence on patient recovery rate[5]. Plants have long been utilized as a source of nourishment by extracting their primary metabolites such as glucose, protein, and fat, but few people realize that their secondary metabolites are also capable of treating human illnesses. Many people underestimate the value of plants as sources of medications.

THEORETICAL REVIEW

The pharmacological potential of the Zingiber genus has been investigated. Ginger, also known as *Zingiber officinale*, is a kind of herbaceous perennial plant that is mostly grown in Maritime Southeast Asia, where the majority of the nations, including Indonesia, Malaysia, and Thailand, have a tropical environment. The chemical elements present in floral parts include largely alkaloids, flavonoids, and caffeoylquinic acids, as well as various oxygenated derivatives such alcohols, aldehydes, and ketones. Zingiberene (23.5%), α-Farnesene (12%), β-sesquiphellandrene (10.3%), ar-curcumene (5.5%), and thirty-five (35) additional secondary metabolites have long been employed as antioxidants, anti-inflammation, anti-microbes, antineoplastic, cardioprotective, anti-obesity, neuroprotective, and anti-diabetic agents.

As a first stage in examining prospective pharmacological effects, molecular modeling and structure-based drug design have shown to be a practical strategy. Drugs were developed through trial and error before to the period of digitalization and technology in computational drug development, which was costly and unsuccessful. CADD (Computer-Aided Drug

Design) is a computational chemistry approach for generating and improving pharmacological activity based on computer calculations, simulations, and predictions between prospective compounds and target receptors. This method will increase drug screening success rates, reduce research mistakes, be cost-effective, and may be completed quickly. Molecular docking is an important first step in CADD because it predicts and calculates the binding energy of ligand-protein complexes

METHODOLOGY

Based on [10], thirty-nine (39) bioactive chemicals have been investigated for the study and are available from PubChem. Receptors are retrieved from Protein Data Bank (PDB) : HMG-CoA Reductase (1HWK), PPAR-γ (4EMA),

PPAR-α (2ZNN), β_1-AR (4AMJ), ACE (2YDM), NPC1L1 (7DFZ), and $P_2Y_{12}R$ (4PXZ) [11].

Preparation of Ligands

All bioactive chemical structures were minimized in energy using ChemDraw3D software and the MM2 force field before being saved in mol2 format.

Preparation of Proteins

All of the proteins in 3D structure were obtained as rough molecules from the Protein Data Bank (PDB). These proteins were created by removing all of the water, cofactor, and ligand molecules in the Ligand preparation portion using the Molegro Virtual Docker program.

Docking Parameters

To permeate all of the docking cavities in the target protein, the docking parameters employed a grid resolution of 0.3 and boosted the docking radius up to 15. The docking technique utilized in this work was MolDock SE (Simplex Evolution), and all of the constrain poses to the cavity, energy minimization, and Hydrogen bonding boxes were checked with a maximum iteration of 1500, a maximum population size of 50, and an energy threshold of 100.

Pharmacokinetics

SwissADME and pkCSM were used to evaluate and compute all elements of pharmacokinetics, beginning with adsorption and progressing through distribution, metabolism, elimination, and toxicity, with multiple factors in each aspect. The in silico SwissADME and pkCSM profiles are useful for predicting pharmacokinetics and toxicological aspects, especially in pre-clinical phases.

Drug-likeness

Lipinski's rule of Five and Veber analysis are used to determine whether bioactive compounds can be taken orally or not, such as Molecular Weight (MW) of less than 500, Octanol/Water Partition Coefficient (AlogP) of less than 5, Hydrogen Bond Acceptor (HBA) of less than 10, Hydrogen Bond Donor (HBD) of less than 5, and Molecular Refractivity of less than 140. If the bioactive compounds do not match the requirements of these parameters, it does not indicate that they cannot be employed as a pharmaceutical candidate. There are numerous methods for modifying it, including the nanoparticles approach.

RESULTS AND DISCUSSION

Molecular Docking Result Analysis

A total of forty (40) bioactive compounds were docked using Molegro Virtual Docker 6.0 to determine the affinity of bonding with related receptors such as HMG-CoA Reductase (PDB: 1HWK), PPAR- (PDB: 2ZNN), 1-AR (PDB: 4AMJ), PPAR- (PDB: 4EMA), ACE (PDB: 2YDM), NPC1L1 (PDB: 7DFZ), and P_2Y_{12} (PDB : 4PXZ). Fourteen (14) bioactive compounds with bond affinity greater than their native and reference ligands were chosen for further

investigation (Table 1). The greatest rerank score was achieved by [10]-Shogaol with -122.347 in score with PPAR-γ. Further, this bioactive compound was displayed in 2D and 3D dimension to analyze and compare the bonding contacts in amino acids residue among the bioactive compound, native and reference ligands with the target proteins.

Table 1 : Molecular docking result of Ginger (*Zingiber Officinale*) bioactive compounds

No	Senyawa bioaktif	Rerank Score						
		1HWK	2ZNN	4EMA	4AMJ	7DFZ	2YDM	4PXZ
1	(+)-Cyclosativene	-63.8483	-72.2671	-65.1263	-65.3441	-69.3405	-66.8409	-59.2436
2	(-)-Camphor	-44.5822	-52.0554	-39.9571	-48.6989	-51.5254	-44.8745	-41.0204
3	(-)-Germacrene D	-70.517	-65.9863	-71.6652	-63.4953	-50.5706	-61.7697	-63.4335
4	(-)-Zingiberene	-77.4075	-77.2296	-80.4528	-78.5679	-70.9917	-68.2957	-84.9434
5	(E)-Nerolidol	-74.4545	-83.9035	-93.5138	-82.0688	-74.2016	**-76.6558**	-85.2041
6	(E,E)-alpha-Farnesene	-83.2434	-79.8222	-91.4529	-80.8123	-74.5269	**-79.8351**	-89.6002
7	(S)-6-Gingerol	**-94.0444**	-89.0678	-105.433	-94.8551	-83.7952	**-87.0908**	-106.245
8	1,8-Cineole	-43.8044	-46.7679	-39.8392	-43.1416	-44.427	-40.4613	-45.0982
9	1-Dehydro-[10]-gingerdione	**-93.2118**	**-106.081**	**-106.583**	-101.083	-102.854	**-111.907**	-129.415
10	**10-Shogaol**	**-90.071**	-93.2553	**-122.347**	-108.89	-97.3484	**-101.782**	-118.272
11	2-Nonanone	-61.369	-60.2325	-62.1272	-69.8235	-54.9648	-58.3113	-70.5278
12	3-Carene	-52.4143	-57.1293	-53.7413	-58.3698	-51.1404	-49.5716	-50.7571
13	4(10)-Thujene	-56.1851	-58.4858	-56.7901	-56.0983	-52.1895	-49.4724	-57.0879
14	4-Terpineol	-58.0115	-58.3489	-58.6711	-63.2557	-58.6553	-50.4198	-58.6982
15	Aframodial	**-87.8818**	-99.9128	-65.6468	-92.4935	-89.9682	**-83.7512**	-82.4288
16	Borneol	-45.3352	-50.1072	-41.3455	-51.6561	-53.4499	-44.9544	-45.367
17	Cedr-8-ene	-69.1492	-63.9295	-56.701	-69.4957	-55.5125	-60.7707	-49.793
18	Citronellol	-66.5824	-63.9676	-70.0285	-68.0943	-60.958	-58.9824	-73.1696
19	Copaene	-61.7125	-61.2024	-61.1124	-57.2074	-53.991	-57.7192	-55.6737
20	Geraniol	-72.8035	-62.2076	-67.5415	-66.9781	-63.8096	-61.0398	-70.6447
21	Gingerenone A	**-102.791**	-101.457	**-115.917**	-111.692	-103.099	**-102.387**	-117.668
22	Isogingerenone B	**-94.3843**	**-106.782**	**-120.573**	-110.565	-104.48	**-105.155**	-121.32
23	Nerol	-62.0357	-65.765	-68.372	-70.1497	-63.0143	-61.6324	-73.9576
24	Nonanol	-58.151	-59.9377	-66.27	-66.1348	-54.9789	-58.407	-66.5711
25	Safrole	-66.5019	-67.9183	-69.7581	-66.4562	-60.351	-57.753	-72.4915
26	Sesquithujene	-74.8735	-75.8783	-82.1845	-77.3312	-70.9876	-72.1002	-87.2919
No	**Senyawa bioaktif**	**Rerank Score**						
		1HWK	**2ZNN**	**4EMA**	**4AMJ**	**7DFZ**	**2YDM**	**4PXZ**
27	Shogasulfonic acid A	**-101.163**	**-109.363**	**-113.271**	-109.617	-109.897	**-118.208**	-129.259
28	Terpinolene	-53.5608	-58.2932	-59.4626	-57.0756	-53.9934	-50.4675	-58.6678
30	Zingiberenol	-82.7071	-79.1815	-79.6515	-84.6912	-71.7372	**-75.4096**	-89.9129
31	Zonarene	-70.3079	-70.3074	-71.1262	-74.8958	-59.2486	-61.1883	-83.4612
32	[6]-Gingerdione	**-88.8025**	-96.4373	-102.81	-96.9494	-87.5943	**-91.7876**	-102.896
33	[6]-Paradol	-80.2621	-88.9077	**-109.2**	-93.9744	-85.1836	**-84.4276**	-92.3931
34	[6]-Shogaol	**-93.356**	-90.3244	-100.404	-100.143	-94.5631	**-89.7248**	-102.257

35	[7]-Paradol	**-93.7929**	-87.4612	-107.098	-101.88	-90.9131	**-93.5463**	-99.2861
36	alpha-Muurolene	-74.1892	-70.8283	-71.8132	-85.0038	-60.2903	-64.8459	-78.0507
37	alpha-Pinene	-45.2548	-46.5639	-41.9697	-46.985	-48.2196	-40.5282	-45.1592
38	beta-Bisabolene	-76.7502	-79.502	-83.9624	-83.3176	-66.2901	-73.2144	-84.9672
39	beta-Cadinene	-64.8074	-66.7039	-63.0567	-59.676	-65.1576	-65.4189	-67.7735
40	beta-Sesquiphellandrene	-71.2004	-80.0038	-85.2125	-78.5707	-66.6949	-72.912	-86.8708
	Native Ligand	-80.5007	-105.931	-105.612	-144.489	-118.976	-73.8388	-180.715
	Senyawa pembanding	-87.2291	-84.7303	-108.067	-115.653	-105.101	-74.6709	-124.404

Bioactive compounds that have rerank score more negative than native and reference ligand

The most negative rerank score of bioactive compounds

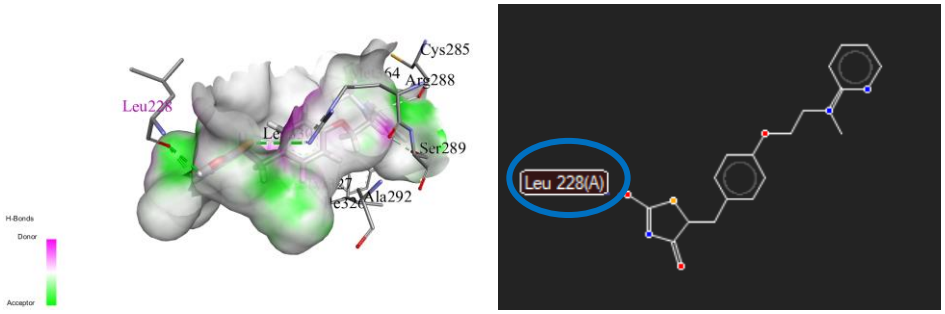

Figure 1 : The 2D/3D interaction diagram of native ligand in the binding site of PPAR-γ (PDB : 4EMA)

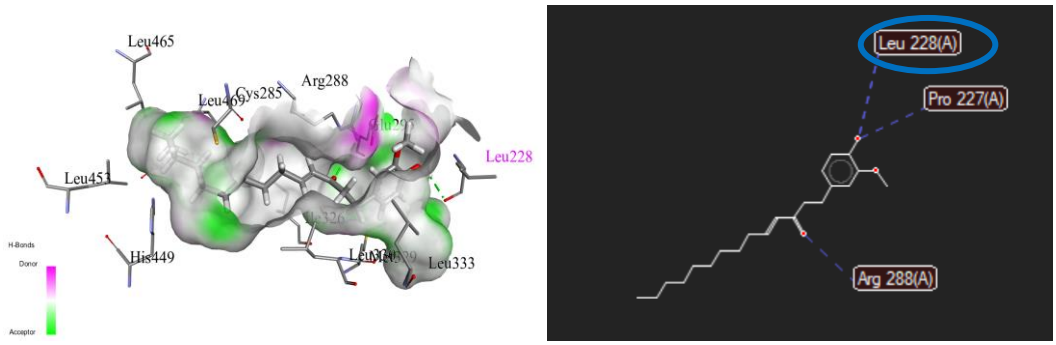

Figure 2 : The 2D/3D interaction diagram of bioactive compound in the binding site of PPAR-γ (PDB : 4EMA)

The bioactive ligand [10]-Shogaol and PPAR-γ complex (fig 1) showed potent binding affinity (Rerank score : -122.347) when compared to the native one (rerank score : -105.612). The native ligand and the bioactive molecule share the same amino acid residue interaction at Leu 228(A), as shown in the figure, indicating that the bioactive compound has the same binding site interaction.

Pharmacokinetics and Toxicology Analysis

Bioactive compounds with a higher rerank score than native and reference ligands will be subjected to pharmacokinetics studies to determine the overall profile in Ginger as well as the most unfavorable profile. There were numerous sections of the pharmacokinetics profile that were studied, such as absorption, distribution, metabolism, and excretion, with distinct parameters in each aspect.

Absorption

Log SW, Log Po/W, and HIA (Human Intestinal Absorption) were the three metrics utilized to determine the absorption profile . The hydrophilicity of the compounds is shown by log SW (not more negative than -6). The hydrophilicity of a substance can influence its solubility. Log Po/W reflects the lipophilicity of the compounds (range 0-3), which is used to estimate the rate of penetration of the compounds into the cell membrane and hence the pharmacological action. Human Intestinal Absorption (HIA) refers to human intestinal lumen's capacity to absorb substances into the bloodstream. The bioavailability of medications may be determined using HIA.

Distribution

The distribution profile was analyzed using three parameters: P-Glycoprotein (P-Gp) substrate, Fraction Unbound (FU), Blood Brain Barrier (BBB), and VDss.

P-Gp, commonly known as ABCB1, is a drug transporter found mostly in hepatocytes. This kind of glycoprotein is responsible for balancing medication concentrations in the human body. By activating this protein (substrate of Glycoprotein-P), xenobiotic substances' bioavailability is reduced, whilst blocking action increases their bioavailability.

The Blood Brain Barrier is a particular barrier present in the brain that prevents chemicals from entering the organ owing to its liveliness. The majority of this barrier is made up of astrocytes, Glia cells, and pericytes. The Log BB (Log Brain Barrier) has a value limit of 0.3; the higher the value, the simpler it is for chemicals to enter the brain (can cause new problem). The Log BB can be calculated with below formula:

$$Log\ BB\ (Brain\ Barrier) = \frac{LogP_{eff} - 0.1725}{2.808}$$

where Log P_{EFF} is a value of membran permeability effectivity which an inversion of resistive effectivity (R_{EFF}).

$$\frac{1}{P_{EFF}} = R_{EFF} = \int_0^z R(z)dz$$

Moreover, R(z) as an integral component reflects the value of resistivity in each membrane, which may also be computed by comparing the Euler exponential to the Boltzmann constant $\left[\frac{1}{K_B T} = \frac{1}{1.38\ x10^{-23}}\ J/K\right]$ and Gibs' energy [$\Delta G =$

$(pK_a - pH)RT\ Ln\ 10]$ with a pH of 7.4, a gas constant of 0.082, T as body temperature in Kelvin, and a coefficient distribution [D(z)] it can also be quantified by $\left[D(z) = \frac{Var\ (z)}{\tau(z)}\right]$ [19,20]

$$R(z) = \frac{e^{\beta(\Delta G(z))}}{D(z)}$$

The fraction unbound (FU) is a predictor of the ratio of free drug to protein-complex drug form. As xenobiotics (drugs) enter the circulation, some of them connect with albumin / globulin (plasm protein), forming a Protein-drug complex (acting as a drug depot), while the remainder are free (known as free-drug) and can interact with target receptors and have clinical effects. The lower the value of Fraction Unbound, the lower the concentration of free medication in the circulation. This circumstance denotes that the chemicals take longer to produce therapeutic effects.

Volume of distribution Steady State (VDss) is an indicator that indicates the pace at which chemicals in the circulation are distributed to target tissue. The figure does not represent the actual volume of the body, but rather the capacity of the medicine to travel from blood to tissue.

$$Vd_{ss} = Vd_s + Vd_p = \left[1 + \frac{K_{12}}{K_{21}}\right] x\ Vd_s$$

$$Vd = Vd_s = \frac{Dose}{Cp^0} = \frac{Dose}{A + B} = \frac{Dose}{K\ x\ AUC^{0-inf}}$$

$$Vdp\ (Perifer\ Volume\ of\ Distribution) = Vd_s\ x\ \frac{K_{12}}{K_{21}}$$

Metabolism

Metabolism analysis included enzymes from many CYP450 subclasses, including CYP1A2, CYP2D6, CYP2C19, CYP2C9, and CYP3A4. These five categories of enzymes are the most significant in drug metabolism, either converting active to inactive form or vice versa.

Excretion

The overall clearance value (L/h/Kg) of excretion analysis reflects how quickly the substances may be removed from the body after being metabolized

Toxicology

The toxicology analysis included LD_{50}, Hepatotoxicity, AMES, and hERG-1 Inhibitor. These characteristics can be used to calculate the toxicity of bioactive substances.

ALT/AST levels in the blood and can result in serious illness such as jaundice, cirrhosis, hepatomegaly, and so on.

Table 2 Absorption and Distribution data of selected bioactive compounds in Ginger (*Zingiber officinale*)

No	Bioactive Compounds	Absorption			Distribution			
		Log Sw	Log Po/w	HIA (%)	P-gp Substrate	BBB permeability (Log BB)	Fraction Unbound (FU)	VDss (L/Kg)
1	(E)-Nerolidol	-3.15	4.19	91.887	No	0.652	0.234	2.3442
2	(E,E)-alpha-Farnesene	-3.37	4.96	93.463	No	0.815	0.145	3.5237
3	(S)-6-Gingerol	-4.58	3.16	92.434	Yes	-0.886	0.207	2.4491
4	1-Dehydro-[10]-gingerdione	-5.93	4.61	91.757	No	-0.468	0.073	2.4491
5	**10-Shogaol**	**-3.39**	**2.24**	**91.312**	**No**	**0.164**	**0.037**	**3.7325**
6	Aframodial	-4.11	3.7	97.783	No	0.197	0.102	1.8323
7	Gingerenone A	-5.63	3.65	91.641	Yes	-0.366	0	1.0495
8	Isogingerenone B	-5.74	3.63	90.825	Yes	-0.474	0.028	1.1641
9	Shogasulfonic acid A	-5.28	2.62	22.839	Yes	1.389	0.033	0.0337
10	Zingiberenol	-2.77	3.79	92.236	No	0.606	0.323	2.6424
11	[6]-Gingerdione	-5.06	3.17	93.34	No	-0.347	0.218	2.0464
12	[6]-Paradol	-5.52	3.96	92.18	No	-0.223	0.147	3.54
13	[6]-Shogaol	-4.8	3.76	92.686	No	-0.197	0.147	3.1696
14	[7]-Paradol	-5.92	4.3	91.836	No	-0.265	0.114	3.8194

Table 3 Metabolism, Excretion and Toxicity profile of bioactive compounds in Ginger (*Zingiber officinale*)

No	Bioactive Compound	Metabolism					Excretion	Toxicity			
		CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor	Total Clearance (L/h/Kg)	LD50 (g/Kg)	AMES toxicity	hERG I Inhibitor	Hepatotoxicity
1	(E)-Nerolidol	No	No	No	No	No	3.2897	351.345	No	No	No
2	(E,E)-alpha-Farnesene	No	No	No	No	No	3.8739	299.781	No	No	No
3	(S)-6-Gingerol	Yes	Yes	Yes	No	No	1.2946	544.934	No	No	No
4	1-Dehydro-[10]-gingerdione	Yes	Yes	Yes	No	Yes	1.9061	671.864	No	No	No
5	**10-Shogaol**	**No**	**Yes**	**No**	**No**	**No**	**2.1785**	**679.298**	**No**	**No**	**No**
6	Aframodial	No	Yes	No	No	No	0.418	661.317	No	No	No
7	Gingerenone A	Yes	Yes	Yes	No	Yes	0.0962	675.022	No	No	No
8	Isogingerenone B	No	Yes	Yes	No	Yes	0.0955	715.613	No	No	No
9	Shogasulfonic	No	No	No	No	No	0.0789	210.4	No	No	No

	acid A							8			
10	Zingiberenol	No	Yes	No	No	No	1.3127	377.14	No	No	No
11	[6]-Gingerdione	Yes	Yes	Yes	No	No	1.6525	559.946	No	No	No
12	[6]-Paradol	Yes	Yes	Yes	No	No	1.5458	586.867	No	No	No
13	[6]-Shogaol	Yes	Yes	Yes	No	No	1.6525	579.35	No	No	No
14	[7]-Paradol	Yes	Yes	Yes	No	No	1.6563	584.918	No	No	No

The most negative rerank score of bioactive compounds

Drug-likeness analysis

A drug-likeness test is required to assess the compounds' suitability for oral drug ingestion. The Lipinski Rule of Five and the Veber test were the two components of this test. Each test assessed many factors such as Molecular Weight, Hydrophilicity, Lipophilicity, and so on.

Lipinski Rule of Five

A crucial metric that was examined is Molecular Weight (MW). At a value less than 500 Da, molecular weight has a substantial link with the rate of permeation and absorption.

Coefficient partition (Log P), also known as Log P/Kow (partition between octanol and water), is a metric that may forecast or assess the ratio of lipophilicity and hydrophilicity, as well as the capability of solubility. The Log P scale ranges from -3 (hyper hydrophilic) to +10. (super lipophilic). The higher the value, the more capable the compounds are of penetrating the membrane; nevertheless, this might result in unstable chemical concentrations throughout the metabolic phase. According to this requirement, the optimal Log P value is less than 5.

$$K_{OW} = \frac{C_O^{Si}}{C_w^{Si}}$$

A hydrogen bond donor (HBD) is a bond or force of attraction that exists between two molecules with opposing polarities. The amount of Nitrogen-Hydrogen bonds and Oxygen-Hydrogen bonds with values less than 5 is used to calculate the Hydrogen Bond Donor.

The term Hydrogen Bond Acceptor (HBA) refers to a group of atoms or molecules that accept Hydrogen bonds, such as oxygen, fluorine, and nitrogen. The bigger the value of HBA, the more lipophilic the chemical, with a value less than 10.

Molecular Refractivity demonstrated the steric characteristic of drugs when they interacted with their receptors. Refractivity is affected by various variables like as temperature, pressure, and the refraction index, and it may be computed using a particular formula.

$$A = \frac{4\pi}{3} N_A \alpha = \left[\frac{M}{\rho}\right]\left[\frac{\varepsilon_r - 1}{\varepsilon_r + 2}\right], [N_A \cong 6.022\ x\ 10^{23}]$$

where ρ is value of molecule density, and ε_r represents of dielectric constant of respective molecule.

The α value represents the average polarizability of the corresponding compounds. Polarizability is the tendency of matter to gain a dipole moment when exposed to an electric environment. It is in charge of the material's dielectric constant as well as its refraction index. In conclusion, the value is a fraction of the dipole moment induction (p) from an atom in an electric environment (E).

$$\alpha = \frac{\|p\|}{\|E\|}$$

Speaking of physics, molecule refractivity has a close association with Lorentz law, thus the mathematical equation of it also may be put in another form.

$$A = \frac{4\pi}{3} N_A \alpha = \frac{n^2 - 1}{n^2 + 2}, [n = refractive\ index]$$

$$n \approx \sqrt{1 + \frac{3Ap}{RT}}$$

Based on Lipinski, a good compound has range of Molecular Refractivity between 40 – 130 [26].

Veber Analysis

In addition to the five Lipinski Rule of Five evaluation categories, there were two (2) important descriptors found by the Veber technique, such as NBR (Number Bond Rotatable) and TPSA (Topology Polar Surface Area).

NBR (Number Bond Rotatable) is a parameter that determines the total number of bonds that may be rotated around themselves (mainly a single bond). The number of NBR and the chemicals' bioavailability are strongly correlated. There are several medications that have more than 12 NBR, such as Protease Inhibitor (HIV drugs) or other pharmaceuticals that have more than 10 NBR but may still be delivered orally with minor dose changes. Veber claims that a compound's optimal NBR value is less than 10.

TPSA (Topology Polar Surface Area) is an aggregation of all polar surface atoms (primarily Oxygen and Nitrogen) (mainly Oxygen and Nitrogen). TPSA is a crucial indication in medicinal chemistry analysis as step to maximize the potential of drugs to permeate to the cell membrane. In general, TPSA is not larger than 140 Å, if the compounds are intended to breach the Blood Brain Barrier then the value must not be greater than 90 Å.

Lipinski's Rule of Five and Veber analysis are not rigid rules that must be followed. There are some medications on the market with molecular weights more than 500 Da, such as Saquinavir, a second-line antiretroviral therapy. These data and analyses will help researchers determine the state of each possible bioactive chemical and take the necessary measures to develop them

Table 4 Lipinski Rule of Five and Veber analysis in Ginger (*Zingiber officinale*) bioactive compounds

No	Bioactive Compounds	Lipinsky Rules					Veber's Rules	
		BM <500 Da	Log P<5	HBA <10	HBD <5	Molar Refractivity	NRB <10	TPSA <140
1	(E)-Nerolidol	222.22	4.3963	1	1	72.4768	7	20.23
2	(E,E)-alpha-Farnesene	204.19	5.2015	0	0	70.9930	6	0
3	(S)-6-Gingerol	294.18	3.2338	4	2	82.7526	10	66.76
4	1-Dehydro-[10]-gingerdione	346.21	5.0830	4	1	100.9338	13	63.6
5	**10-Shogaol**	**332.24**	**4.5994**	**3**	**1**	**99.7368**	**9**	**46.53**
6	Aframodial	318.22	4.1024	3	0	90.0810	5	46.67
7	Gingerenone A	356.16	3.8057	5	2	100.1066	9	75.99
8	Isogingerenone B	386.17	3.8143	6	2	106.6586	10	85.22
9	Shogasulfonic acid A	438.13	3.9768	8	3	110.9644	11	138.74
10	Zingiberenol	222.2	4.0861	1	1	70.3168	4	20.23
11	[6]-Gingerdione	292.17	3.4420	4	1	81.7528	10	63.6
12	[6]-Paradol	278.19	4.2630	3	1	81.3628	10	46.53
13	[6]-Shogaol	276.17	4.0390	3	1	81.2688	9	46.53
14	[7]-Paradol	292.2	4.6531	3	1	85.9798	11	46.53

The most negative rerank score of bioactive compounds

Molecular Dynamic Analysis

Molecular Dynamic is a scientific method for testing chemicals as near to the human body as possible by adding physiological pH (7.4) and temperature (37^0C / 310K) for 5000 ps (5 ns). This study chose the most negative candidate in rerank score to be studied in Molecular Dynamic, namely [10]-Shogaol with PPAR- as the target enzyme (PDB : 4EMA). This method tested four (4) cycles including empty protein (receptor), receptor-native ligand complex, receptor-reference ligand complex, and receptor-test ligand complex with a few parameters such as Total Energy, RMSD (Root Mean Square Deviation) both in protein and ligands, RMSF (Root Mean Square Fluctuation), and (RG) Radius of Gyration. [27,28].

Total Energy

Total Energy represents the variation of energy throughout time. This energy fluctuation can indicate the strengthening and relaxation of receptor-ligand interactions. The research of [10]-Shogaol revealed that there was an energy fluctuation to obtain the equilibrium state between 0 and 0.5 ns. As can be seen in the figure, there is no substantial difference in energy between empty protein and protein-ligand complex. The bioactive molecule [10]-Shogaol has the highest energy rate, with a value of -662338.393 Kj/mol.

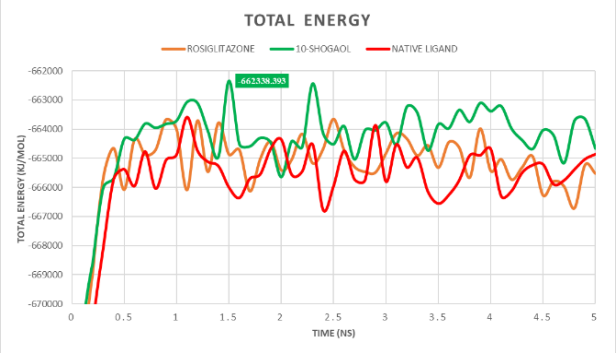

Figure 3 : Total Energy of simple protein and its complex with PPAR-γ

RMSD

Root Mean Square Deviation (RMSD) is a measure that describes how a protein's structural structure varies after interacting with a ligand. RMSD has a standard value of no more than 3 Å. There is also Ligand RMSD, which is similar to protein RMSD which we can detect the fluctuation / movement of the ligand over time. The diagram demonstrated that the fluctuation of protein RMSD was less than 3 Å, with the highest point for protein-test ligand complex at 1.394 Å and the lowest point at 0.452 Å, indicating that the bond between the test ligand and the target protein is stronger than the bond between the target protein and other complexes.

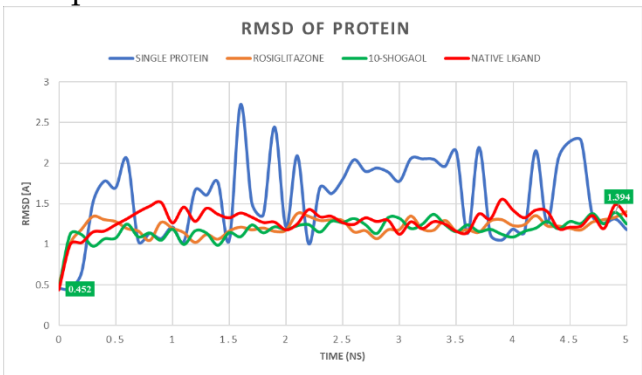

Figure 4 : RMSD of Protein value of single protein and its complex

It may be determined that the test chemical [10]-Shogaol had the best RMSD value, with the lowest RMSD value range at 0.139 Å and the maximum point at 2.266 Å.

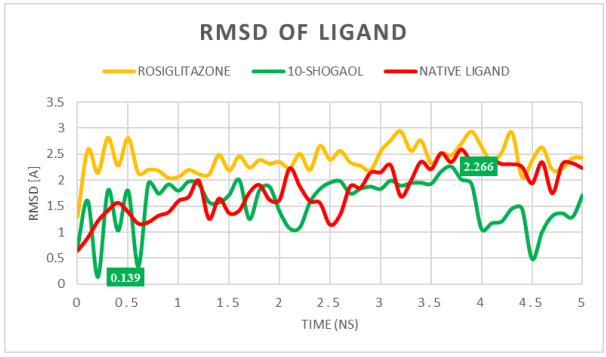

Figure 5 : RMSD of Ligand value of single protein and its complex

RMSF

Root Mean Square Fluctuation (RMSF) is a parameter that is comparable to RMSD. The distinction between RMSD and RMSF is that RMSF can observe and evaluate interaction variation at the level of amino acids and even atoms. This characteristic will allow us to precisely determine the activity of the protein and ligand. RMSF has a standard value of no more than 3

Å. The tested ligands had lower RMSF values than either the single protein or the natural ligand complex, as shown in the diagram. Research has also shown that the evaluated bioactive compounds outperformed the reference and native ligands in terms of activity.

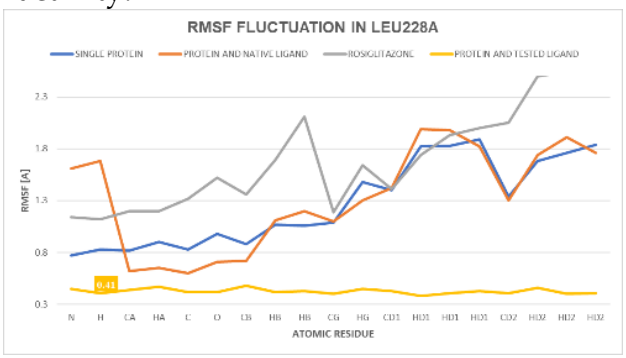

Figure 6 : RSMF Fluctuation in Leu 288 (A)

Radius of Gyration (RG)

The radius of angular dynamic movement of protein (either alone or in association with ligand) with the solvent is described by the Radius of Gyration indicator. RG can be used to determine the solubility of a chemical. A high RG value implies that the drug can interact with proteins and receptors (protein unfolded) and vice versa.

In general, the diagram shows that protein complex linkages have a narrower range of values when compared to single proteins. In particular, the native ligand had no effect on the Radius Of Gyration value, but the reference and test ligands appeared to alter the conformation of the target protein / receptor. The average Radius of Gyration of the test ligands was 19,108 nm, while the native and reference ligands were 19.054 nm and 19.088 nm, respectively.

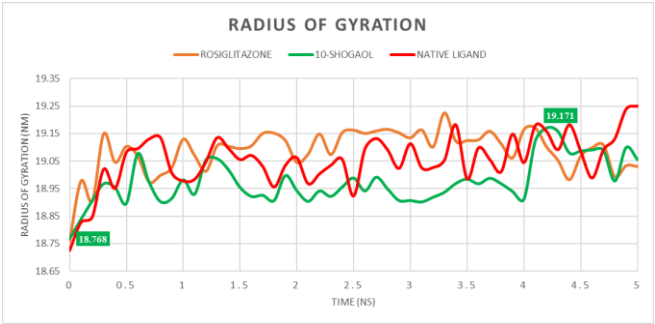

Figure 7 : Radius of Gyration value of Single Protein and its complex

CONCLUSIONS AND RECOMMENDATIONS

According on the findings of the in silico tests, one of the bioactive compounds reported in Ginger, namely [10]-Shogaol, has stronger activity than the reference and native ligands against PPAR-γ, suggesting that this bioactive molecule can affect the activity of the enzyme more effectively.

The Molecular Docking investigation revealed a favorable outcome, with the tested bioactive molecule outperforming the reference and native ligands in terms of value. Furthermore, the pharmacokinetics and toxicology analyses, as well as the drug-likeness test, revealed a good outcome for the bioactive molecule (Lipinski Rule of Five and Veber analysis).

The Molecular Dynamic study also yielded a good result, with all metrics such as Total Energy, Radius of Gyration, RMSD for both protein and ligand, and RMSF being higher than the native and reference ligands.

FURTHER STUDY

This study will go in-depth into the structural analysis of compounds in ginger (Zingiber officinale) through an in silico approach. Using computational methods, we will investigate the interactions of key compounds with Acute Coronary Syndrome (SCS)-related targets, expanding the understanding of ginger's potential as a reliable source of bioactive compounds.

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