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Original Research Article

In Silico and *In Vitro* Study of The Ethanol Extract of The White Garland Lily (*Hedychium coronarium* J. Koenig) as a Tyrosinase Inhibitor

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ABSTRACT

The Zingiberaceae family includes the White Garland Lily (*Hedychium coronarium* J. Koenig). It is used in traditional medicine for fever, analgesics, and indigestion. In the current study, *in silico* prediction of the active compound from *Hedychium coronarium*, analysis of the interaction with a tyrosinase inhibitor, and testing of the tyrosinase inhibitor using dopachrome compounds were done. The software used for docking simulation is Autodock-GPU. Kojic acid solution containing 1000, 500, 250, 125, and 62.5 ppm was measured into a 96-well microtiter dish to which tyrosinase enzyme solution was added, and the pH was adjusted with phosphate buffer. The mixture was incubated at 37ºC for 30 minutes before the absorbance was measured at a wavelength of 492 nm using a microplate reader. Triparanol molecules have the best bonding affinity in the molecular docking simulation data, with a -8.00 kcal/mol value. The extract yield was 3.88%, total ash content of 12.60% ± 0.27%, acid-insoluble ash content of 0.23% ± 0.02%, and water content of 5.96%. *Hedychium coronarium* has the potential to be developed as a Tyrosinase inhibitor from the result of the *insilico* investigation. Based on the result, the ethanol extract inhibited the tyrosinase enzyme with an IC_{50} value of 183.85 ppm compared to kojic acid with an IC_{50} value of 17.09 ppm.

Keywords: *In silico, In vitro, Hedychium coronarium J. Koenig, Tyrosinase*

Introduction

Indonesia is located in a tropical region with constant sun exposure. Sunlight, specifically UV radiation, stimulates the synthesis of the pigment melanin, causing the skin to darken.[1] Many skin lightening and whitening products are now available for the clinical treatment of pigmentary disorders such as hyperpigmentation following inflammation. However, whiteners frequently contain mercury, hydroquinone, and kojic acid.[2] These compounds are carcinogenic and cause skin irritation, redness, heat, and even itching.[3]

Whitening agents work through various mechanisms, such as tyrosinase inhibitors (tyrosinase enzyme maturation inhibitors) or by inhibiting the movement of pigment granules (melanosomes) from melanocytes to surrounding keratinocytes. Tyrosinase is an enzyme that synthesizes melanin in melanocytes.[4] It converts L-tyrosine to L-DOPA monophenolase and L-DOPA to dopaquinone. Furthermore, dopaquinone is converted to melanin through chemical reactions, which darkens the skin.[5] Inhibiting tyrosinase activity reduces melanin synthesis and creates skin shadows.[6] Inhibition of the tyrosinase enzyme could prevent skin hyperpigmentation. As stated earlier, organic sources like plants and microorganisms, as well as their active compounds, possess remarkable potential as natural sources for inhibiting tyrosinase.[7]

Flavonoids, tannins, and saponins are examples of compounds that can inhibit tyrosinase activity. In addition, phenolic compounds strongly inhibit the tyrosinase enzyme and monophenolase and diphenolase activities.[8]

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Hedychium coronarium is one of the plants that can inhibit tyrosinase. The extract of *Hedychium coronarium* contains flavonoid and phenolic compounds that could be used as an antioxidant and tyrosinase inhibitor.[9] Flavonoid compounds inhibit the tyrosinase enzyme through competitive inhibition of L-DOPA oxidation by the tyrosinase enzyme, which acts as a copper (Cu) chelator of the tyrosinase enzyme structure.[10]

Hedychium coronarium is a plant in the Zingiberaceae family that grows in tropical and subtropical regions such as the Himalayas, Asia, and the Pacific Islands. It is known as *gandasuli* in Indonesia and is used as a traditional medicine to treat various diseases. For example, it is used as a stimulant in Indonesia and China and as a smoothing agent for indigestion in Malaysia. In addition, it is used as a fever reducer in Thailand and as a treatment for muscle pain in India.[11] *Hedychium coronarium* extract has antimicrobial, anti-inflammatory, antioxidant, and cytotoxic activity.[12]

Hedychium coronarium contains phenolics, flavonoids, diterpenes, sesquiterpenes, fatty acids, and steroids. Five compounds were obtained from high-resolution liquid chromatography-mass spectrometry (HRLC-MS/MS) on Hedychium coronarium rhizomes, namely 3-Hydroxy suberic acid, Digoxigenin monodigitoxoside, Ginkgolide C, Swietenine, and Triparanol.[13] Melanocytes produce two types of melanin: eumelanin, which gives brown or black pigment, and pheomelanin, which gives red or yellow pigment.[14] The activation or inhibition of signalling pathways related to melanogenesis, such as protein kinase A and mitogen-activated protein kinase, can be influenced by inflammatory factors that bind to receptors. This, in turn, can affect the expression of genes related to melanogenesis and regulate the processes involved in skin pigmentation.[15] The enzyme tyrosinase catalyzes the hydrolysis of L-tyrosine to DOPA and the oxidation of DOPA to dopaquinone. The TRP-1 enzyme oxidizes DHICA to produce eumelanin. Dopachrome spontaneously disappears without the carboxylic acid group to form 5,6-dihydroxyindole (DHI).[16] This study aims to predict active compounds from *Hedychium coronarium* J. Koenig and analyze interactions with a tyrosinase inhibitor using *in silico* methods.

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Material and Methods

In Silico Analysis

The receptor used in this study is the crystal structure of the human tyrosinase-related protein in complex with mimosine with PDB ID: 5M8N.[17] Five compounds were analyzed *in silico*, namely 3-Hydroxysuberic acid, Digoxigenin monodigitoxoside, Ginkgolide C, Swietenine, and Triparanol.[13] Following Avogrado software preparation, geometry optimization is performed using the Molecular Mechanics method, Merck Molecular Force Field (MMFF94).[18,19]. The software used for docking simulation is Autodock-GPU https://github.com/ccsb-scripps/AutoDock-GPU.[20] The grid box was prepared using a Python-based script. Redocking to calculate RMSD. Amino acid interaction analysis was carried out using Biovia Discovery Studio software https://discover.3ds.com/.[21,22] Protein interaction analysis was conducted to see the network of compounds with proteins using STITCH http://stitch.embl.de/.[23]

Tools

The following items were used in this study: an extract vessel, filter paper, rotary evaporator (Bhuci®), capillary tube, silica gel TLC plates GF254, drop pipette, spatula, Erlenmeyer (Pyrex®), porcelain crucible, volumetric flasks of 10 mL (Pyrex®), and 100 mL (Pyrex®), funnel (Pyrex®), measuring cup (Pyrex®), micropipette (Eppendorf®), analytical balance (Kern Abj®), desiccator, vapour cup, oven (Kirin®), pH meter (Hanna®), 96 well-microtiter plate (Iwaki®), furnace, microplate spectrophotometer (xMarkTM), and vials.

Reagents

The following reagents were used: ethanol 96%, aqua dest, aqua bidestalata, dimethyl sulfoxide (DMSO) (Merck®), kojic acid (Sigma Aldrich®), tyrosinase enzymes (Sigma Aldrich®), L-DOPA (Sigma Aldrich®), $FeCl_3$(Merck®), $NaH_2PO_4.2H_2O$ (Merck®), Na_2HPO_4 (Merck®), Sitroborat, Dragendorf reagent, Libermann-Burchad reagent.

Hedychium coronarium J. Koenig Extract Test as Tyrosinase Inhibitors

The dried rhizome of *Hedychium coronarium J. Koenig* was ground and extracted with ethanol. The extract was concentrated to drying using a rotary evaporator at 40℃. A 100 mg extract containing 1000, 500, 250, 125, and 62.5 ppm was measured into a 96-well microtiter dish. Afterward, 20 µL of tyrosinase enzyme solution (250 units/mL) was added, and it was made up to 100 mL by adding 30 µL of phosphate buffer pH 6.5. This was followed by incubating for 5 minutes at room temperature. Then, 100 µL of a 5.07 mM L-DOPA substrate solution was added. Next, the mixture was incubated at 37ºC for 30 minutes, and the absorbance was measured at a wavelength of 492 nm using a microplate reader.

Preparation of Kojic Acid Solution

Powder kojic acid (10 mg) was carefully weighed and dissolved in DMSO to a volume of 10 mL in a measuring flask. Next, dilution was carried out to obtain various concentrations of the kojic acid solution, which are 1000, 500, 250, 125, and 62.5 ppm.

Results and Discussion

The organoleptic properties of the ethanolic extract of *Hedychium coronarium* J. Koenig are thick extract, blackish-brown colour, characteristic aromatic odour, and bitter taste. The total ash content is 12.60 ± 0.27%, acid insoluble ash content 0.23 ± 0.02%, and 5.96% water content. Interaction networks of chemicals and proteins analysis were performed to predict target receptors.[23] From the analysis results, only two compounds had specific targets, namely Ginkgolide C and Triparanol. The results of the specific target analysis can be seen in Table 1. Redocking was carried out to validate the ability of the docking software to predict the bond and compare it with the native ligands from crystallography. The Root Mean Square Deviation (RMSD) score in redocking was 1.78. It shows that the docking software used is similar to the crystallographic results. The results of molecular docking between compounds and target proteins (PDB ID: 5M8N) are shown in Table 2. Figure 1 shows the type of interaction between the compounds and protein targets.

The specific target of Ginkgolide C is the platelet-activating factor receptor (Figure 1). The platelet-activating factor receptor (PAFR) is a seven-transmembrane G-protein-coupled receptor that plays a role in various pathological and physiological processes.[24] The target enzymes of Triparanol are 7-dehydrocholesterol reductase, 24-dehydrocholesterol reductase, Emopamil binding protein (sterol isomerase), Sigma non-opioid intracellular receptor 1, and Rab geranylgeranyltransferase. 7-dehydrocholesterol reductase is a component of the cholesterol biosynthesis process, lowering the C7-C8 double bond in the two intermediates of that route, cholesta-5,7,24-trien-3 beta-ol and 7-dehydrocholesterol/7-DHC.[25] The 24-dehydrocholesterol reductase is a reduction of the delta-24 double bond of sterol intermediates during cholesterol biosynthesis. It is catalyzed by the flavin adenine dinucleotide (FAD)-dependent oxidoreductase encoded by this gene. A leader sequence in the protein leads it to the endoplasmic reticulum membrane.[26] Emopamil binding protein (sterol isomerase) is an endoplasmic reticulum membrane protein that develops oligodendrocytes, autophagy, and cholesterol production. In addition, an inborn disease called Conradi-Hunermann syndrome may be induced by the mutation of EBP.[27] Sigma non-opioid intracellular receptor 1 is a potential therapeutic target for long QT syndrome. Rab geranylgeranyltransferase, which catalyzes the attachment of two geranylgeranyl groups to the C-terminal cysteine residues of Rab proteins, is essential for membrane connection and the activity of these proteins in intracellular vesicular trafficking.[28] In all the target predictions, no direct mechanism of the tyrosinase enzyme was found.

Table 1: Prediction of interaction for each compound from *Hedychium coronarium*

Compound	Predicted Functional Partner	Score
3-Hydroxy suberic acid	None	None
Digoxigenin monodigitoxoside	None	None
Ginkgolide C	Platelet-activating factor receptor	0.800
	Vesicle-associated membrane protein 7	0.429
Swietenine	None	None
Triparanol	7-dehydrocholesterol reductase	0.927
	24-dehydrocholesterol reductase	0.884
	Emopamil binding protein (sterol isomerase)	0.694
	Sigma non-opioid intracellular receptor 1	0.645
	Rab geranylgeranyltransferase, alpha subunit	0.437

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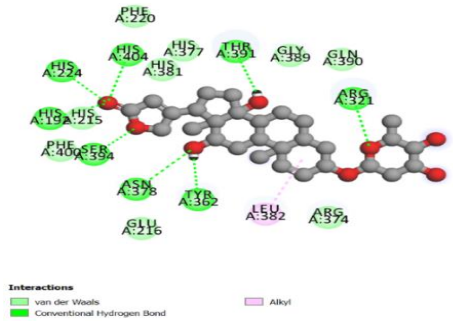
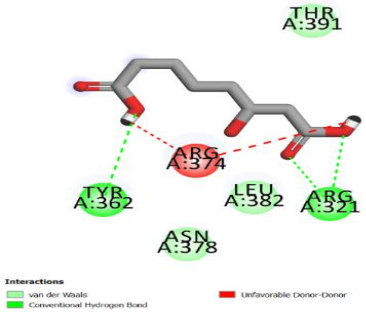
Table 2: Analysis of the affinity and interaction

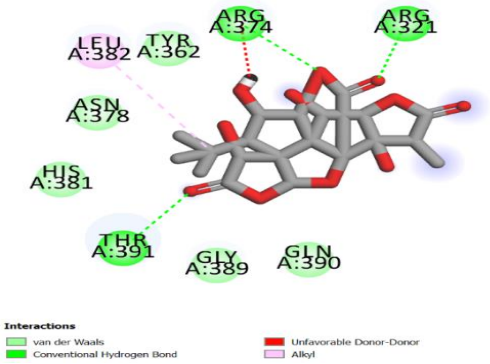
Active Compound	Affinity (kcal/mol)	Interaction
3-Hydroxy suberic acid	-4.18	Hydrogen Bond: TYR362, ARG321
Digoxigenin monodigitoxoside	-7.73	Hydrogen Bond: TYR362, ASN378, SER394, HIS192, HIS224, HIS404, THR391, ARG321 Alkyl: LEU382
Ginkgolide C	-5.32	Hydrogen Bond: ARG321, ARG374, THR391 Alkyl: LEU382
Swietenine	-6.91	Hydrogen Bond: HIS392 Carbon Hydrogen: GLU216, HIS215
Triparanol	-8.00	Pi-Lone pair: THR39 Carbon Hydrogen Bond: VAL211, GLU216, ASP211



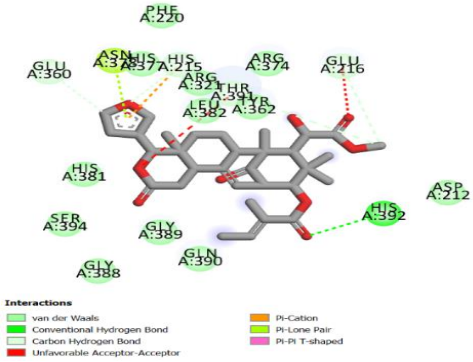
a) 3-Hydroxy suberic acid



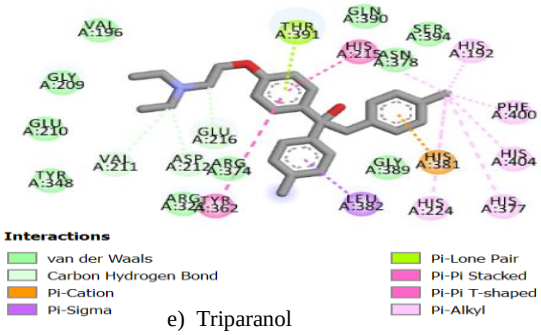
b) Digoxigenin monodigitoxoside



C) Ginkgolide C



d) Swietenine



e) Triparanol

Figure 1: Interaction of compound with human tyrosinase (PDB ID: 5M8N)

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Molecular docking is a computational method to see the interactions and binding between ligands and receptors.[29,22] Triparanol molecules have the best binding affinity in the molecular docking simulation data, with a value of -8.00 kcal/mol compared to the native ligand mimosine, with a docking score of -5.86 kcal/mol. It was an interaction of a hydrogen bond with an amino acid in TYR362 and ARG321 when engaged with 3-hydroxysuberic acid. Hydrogen bonds can be found in digoxigenin monodigitoxoside at the following residues: TYR362, ASN378, SER394, HIS192, HIS224, HIS404, THR391, and ARG321. Moreover, there is an alkyl bond in the LEU382 residue.

A hydrogen bond exists between the residues ARG321, ARG374, and THR391 of ginkgolide C. The amino acid LEU382 also contains a specific kind of alkyl bond. The HIS392 amino acid of swietenine contains a hydrogen link, whereas the GLU216 and HIS215 amino acids each contain a carbon-hydrogen bond. In triparanol, the THR39 amino acid has a Pi-Lone pair link, while the VAL211, GLU216, and ASP 211 amino acids have a Carbon Hydrogen Bond type bond.

The *in vitro* test results showed that the IC_{50} of the ethanolic extract of the rhizome of *Hedychium coronarium* J. Koenig was 183.85 ppm. That is, at a concentration of 183.85 ppm, the ethanolic extract of *Hedychium coronarium* J. Koenig rhizome inhibited 50% of the tyrosinase enzyme activity (Figure 2). The smaller the IC_{50} value of the test substance, the more potent the activity as a skin-whitening agent. The IC_{50} value of *Hedychium coronarium* J. Koenig extracts was computed from 21.073ln(x)-59.87; an equation was derived from the plot of percentage inhibition against extract concentration (µg/mL). This result was about 11 times lower than kojic acid, whose IC_{50} = 17.09 ppm (Figure 3). The study showed that the Hedychium coronarium J. Koenig extract exhibited better inhibitory activity at lower concentrations. The difference between the IC_{50} values of kojic acid and *Hedychium coronarium* J. Koenig rhizome extract could be that kojic acid is a pure compound with a competitive mechanism of action on the tyrosinase enzyme.

Conclusion

One of the active compounds from *Hedychium coronarium* J. Koenig was triparanol. It has a binding affinity in the molecular docking simulation data, with a value of -8.00 kcal/mol. STITCH analysis has functional partners in ginkgolide C and triparanol. Triparanol, an active compound in *H. coronarium*, formed a Pi-lone pair with THR39 amino acid and a carbon-hydrogen bond interaction with VAL211, GLU216, and ASP211 amino acids. In conclusion, the ethanolic extract of *H. coronarium* rhizome has tyrosinase enzyme inhibitory activity. It may be further explored as a pharmaceutical lead for developing a natural agent for skin hyperpigmentation for use in cosmetics and managing related disorders

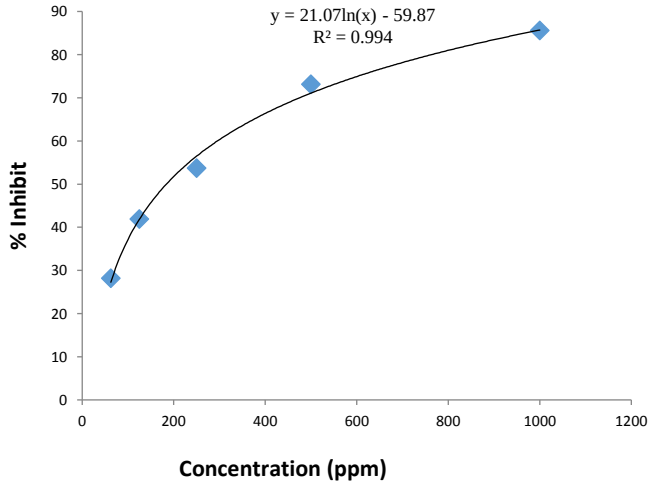

Figure 2: The curve of tyrosinase inhibitor enzyme rhizome extract *Hedychium coronarium* J. Koenig

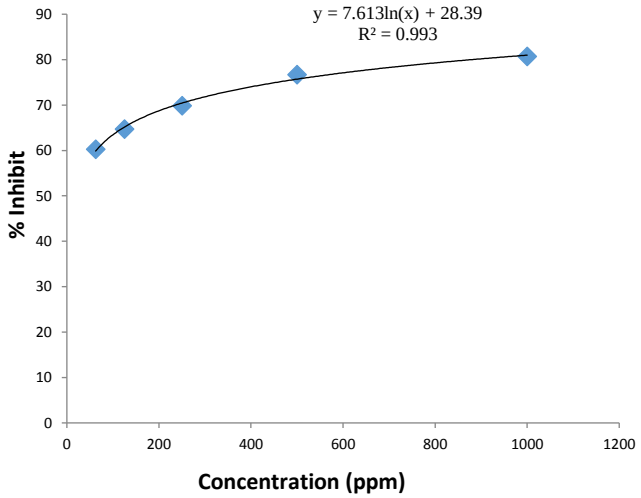

Figure 3: Tyrosinase enzyme inhibitor curve from kojic acid compound

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Solano F. Photoprotection and skin pigmentation: Melanin-related molecules and some other new agents obtained from natural sources. Molecules. 2020;25(7):1-18
2. Couteau C, Coiffard L. Overview of skin whitening agents: Drugs and cosmetic products. Cosmetics. 2016;3(3):1-16
3. Agorku ES, Kwaansa-Ansah EE, Voegborlo RB, Amegbletor P, Opoku F. Mercury and hydroquinone content of skin toning creams and cosmetic soaps, and the potential risks to the health of Ghanaian women. Springerplus. 2016;5(1):1-5
4. Talmadge JE, Cowan KH. Gene Therapy in Oncology. Abeloff's Clin Oncol Fifth Ed. 2014;493-507.e4.
5. Pillaiyar T, Manickam M, Namasivayam V. Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. J Enzyme Inhib Med Chem. 2017;32(1):403–25.
6. Li Q, Yang H, Mo J, Chen Y, Wu Y, Kang C, Sun Y, Sun H. Identification by shape-based virtual screening and evaluation of new tyrosinase inhibitors. PeerJ. 2018;2018(1):1-14
7. Zolghadri S, Bahrami A, Hassan Khan MT, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, Saboury AA. A comprehensive review on tyrosinase inhibitors. J Enzyme Inhib Med Chem. 2019;34(1):279–309.
8. Panzella L, Napolitano A. Natural and bioinspired phenolic compounds as tyrosinase inhibitors for the treatment of skin hyperpigmentation: Recent advances. Cosmetics. 2019;6(4):1-13

9. Pachurekar P, Dixit A. A Review on Pharmacognostical Phytochemical and Ethnomedicinal Properties of *Hedychium Coronarium* J. Koenig an Endangered Medicine. Int J Chinese Med. 2017;1(2):49–61.
10. Solano F. On the metal cofactor in the tyrosinase family. Int J Mol Sci. 2018;19(2):1-17
11. Tavares WR, Barreto M do C, Seca AML. Uncharted Source of Medicinal Products: The Case of the Hedychium Genus. Medicines. 2020;7(5):1-23.
12. Rachkeeree A, Kantadoung K, Suksathan R, Puangpradab R, Page PA, Sommano SR. Nutritional Compositions and Phytochemical Properties of the Edible Flowers from Selected Zingiberaceae Found in Thailand. Front Nutr. 2018;5(1):1-10
13. Panigrahy SK, Kumar A, Bhatt R. Hedychium coronarium Rhizomes: Promising Antidiabetic and Natural Inhibitor of α-Amylase and α-Glucosidase. J Diet Suppl. 2020;17(1):1–7.
14. Hirt PA, Paus R. Healthy Hair (Anatomy, Biology, Morphogenesis, Cycling, and Function). Alopecia. 2019;1–22.
15. Fu C, Chen J, Lu J, Yi L, Tong X, Kang L, Pei S, Ouyang Y, Jiang L, Ding Y, Zhao X, Li S, Yang Y, Huang J, Zeng Q. Roles of inflammation factors in melanogenesis (Review). Mol Med Rep. 2020;21(3):1421–30.
16. Kobayashi T, Imokawa G, Bennett DC, Hearing VJ. Tyrosinase stabilization by Tyrp1 (the brown locus protein). J Biol Chem. 1999;273(48):31801–5.
17. Lai X, Wichers HJ, Soler-Lopez M, Dijkstra BW. Structure of Human Tyrosinase Related Protein 1 Reveals a Binuclear Zinc Active Site Important for Melanogenesis. Angew Chemie - Int Ed. 2017;56(33):9812–5.
18. Hanwell MD, Curtis DE, Lonie DC, Vandermeerschd T, Zurek E, Hutchison GR. Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. J Cheminform. 2012;4(8):1-17
19. Halgren TA. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. J Comput Chem. 1996;17(5–6):490–519.
20. Santos-Martins D, Solis-Vasquez L, Tillack AF, Sanner MF, Koch A, Forli S. Accelerating A uto D ock 4 with GPUs and Gradient-Based Local Search. J Chem Theory Comput. 2021;17(2):1060–73.
21. Putra PP, Fauzana A, Lucida H. In Silico Analysis of Physical-Chemical Properties, Target Potential, and Toxicology of Pure Compounds from Natural Products. Indones J Pharm Sci Technol. 2020;7(3):107-117.
22. Asnawi A, Nedja M, Febrina E, Purwaniati P. Prediction of a Stable Complex of Compounds in the Ethanol Extract of Celery Leaves (*Apium graveolens* L.) Function as a VKORC1 Antagonist. Trop J Nat Prod Res. 2023;7(2):2362–70.
23. Szklarczyk D, Santos A, Von Mering C, Jensen LJ, Bork P, Kuhn M. STITCH 5: Augmenting protein-chemical interaction networks with tissue and affinity data. Nucleic Acids Res. 2016;44(D1):D380–4.
24. Howard KM. Platelet-Activating Factor Receptor. Encycl Biol Chem Second Ed. 2013;533–7.
25. Luu W, Hart-Smith G, Sharpe LJ, Brown AJ. The terminal enzymes of cholesterol synthesis, DHCR24 and DHCR7, interact physically and functionally. J Lipid Res. 2015;56(4):888–97.
26. Lu P, Jiang Y, Xia Z. Hsa_circ_0003221 facilitates the malignant development of bladder cancer cells via resulting in the upregulation of DHCR24 by targeting miR-892b. Investig Clin Urol. 2022;63(5):577–88.
27. Long T, Hassan A, Thompson BM, McDonald JG, Wang J, Li X. Structural basis for human sterol isomerase in cholesterol biosynthesis and multidrug recognition. Nat Commun. 2019;10(1):1-8
28. Song L, Bekdash R, Morikawa K, Quejada JR, Klein AD, Aina-Badejo D, et al. Sigma non-opioid receptor 1 is a potential therapeutic target for long QT syndrome. Nat Cardiovasc Res. 2022;1(2):142–56.
29. Putra PP, Armin F, Florida N, Yusuf GV, Suharti N. Molecular Dynamics, Prediction of Toxicity, and Interaction of the Active Compound *Caesalpinia sappan* on Essential Lipids Klebsiella pneumoniae. Adv Heal Sci Res. 2021;302-309